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STUDIES IN SERUM ELECTROLYTES. XVI. CHANGES IN THE SERUM AND BODY FLUIDS IN ANOREXIA NERVOSA 209
F. William Sunderman and Edward Rose

A DIURNAL RHYTHM IN THE EXCRETION OF NEUTRAL REDUCING LIPIDS BY MAN AND ITS RELATION TO THE 17-KETOSTEROID RHYTHM 221
Gregory Pincus, Louise P. Romanoff and James Carlo

EFFECTS OF STARVATION ON SEX HORMONES IN THE MALE 227
Eugene C. Jacobs

THE RAPID RAT TEST FOR PREGNANCY. THE OVARIAN HYPEREMIA RESPONSE AS A ROUTINE DIAGNOSTIC PROCEDURE 233
Gardner M. Riley, Marjorie H. Smith and Pearl Brown

ADRENAL CORTICAL HORMONE EXCRETION IN ENDOCRINE AND NON-ENDOCRINE DISEASE AS MEASURED BY CHEMICAL ASSAY . . . 244
William H. Daughaday, Herbert Jaffe and Robert H. Williams

RELATION OF OBESITY TO THE FUNCTION OF THE THYROID GLAND, ESPECIALLY AS INDICATED BY THE PROTEIN-BOUND IODINE CONCENTRATION IN THE PLASMA 257
Robert H. Williams

STUDIES ON ANTIHORMONE SPECIFICITY WITH PARTICULAR REFERENCE TO GONADOTROPIC THERAPY IN THE FEMALE 262
J. H. Leathem and A. E. Rakoff

ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION 269

ANNOUNCEMENT OF THE 1948 LAURENTIAN HORMONE CONFERENCE . . 270

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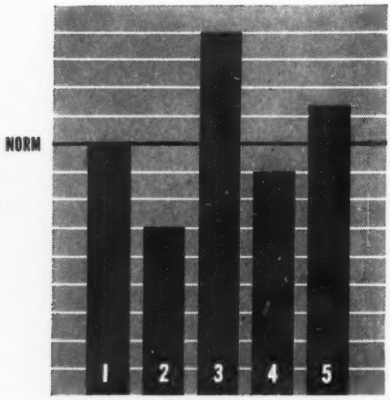

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STUDIES IN SERUM ELECTROLYTES. XVI. CHANGES IN THE SERUM AND BODY FLUIDS IN ANOREXIA NERVOSA

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THE purpose of this paper is to report studies of changes in composition and distribution of the body fluids and serum electrolytes; assays of urinary hormones; and observations on chloride metabolism in a patient suffering from anorexia nervosa, whom we have followed for a period of three years. Wherever possible, our data are compared to those recently reported (1) pertaining to a severely emaciated individual who had undergone a voluntary fast for religious reasons for a period of 45 days. Such comparison would seem appropriate since the data might be regarded as pertaining to chronic and acute undernutrition, respectively.

The syndrome of anorexia nervosa has been so extensively described that no attempt will be made to review the literature or to repeat the clinical description of the disorder.

CASE REPORT

The patient, F. J., is a white, married woman, 29 years of age. At the age of 17 she weighed 112 pounds but became obsessed with the idea that she was overweight. She then began to reduce her caloric intake. During the next nine years her weight gradually fell to a minimum level of 56 pounds. She insisted that throughout this period her

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appetite remained normal and that she enjoyed her meals. For several years she had noted increasing constipation with much indigestion, characterized by flatulence and vague, generalized abdominal discomfort. During the past few years she frequently induced vomiting after meals. Although she complained of increasing weakness and fatigability, this was belied by her sprightly behavior during her hospital admissions.

The patient's menses began at the age of 17 but were very scanty and after a few months they ceased entirely for about two years. Scanty, irregular menstruation then recurred for about two years, but amenorrhea has been complete for the past six years. The patient married at the age of 21 but never became pregnant. Her libido was always minimal and for the past several years she has been completely frigid. Dyspareunia has been increasingly severe.

FIGURE 1

The patient's personality has been definitely abnormal. Although she talked at length about her complaints, she was vague regarding dates and time relationships. She quarreled with many of her physicians and has been bitterly critical of the various hospitals where she had stayed. During each of her several admissions to the medical wards of the Hospital of the University of Pennsylvania she quarreled with the nursing and dietetic staffs and with her fellow patients. She enjoyed the attention of students and physicians and was always eager to be presented at clinical lectures or to be the subject of laboratory tests. She had baffled the best efforts of several psychiatrists to discover the real basis of her behavior. From time to time she exhibited anxiety regarding the possible existence of organic diseases such as brain tumor, cholecystitis, or intestinal obstruction.

On physical examination the patient's appearance was one of striking emaciation without commensurate muscular weakness (Figure 1). She was 61 inches in height and weighed 56 pounds. Cranial, axillary, and pubic hair were normal in amount. The hair was somewhat dry. Hypertrichosis was present on the face, forearms, and back. The skin was dry and wrinkled with some scaling. Her blood pressure measurements averaged 110/80 mm. of Hg. The patient's pulse rates were normal but her oral temperatures were subnormal throughout most of the period of our observations, recordings between 96° and 97° F. being obtained almost daily. The mucous membranes of the mouth and pharynx were pale and smooth. Dental caries was widespread and she had only four teeth remaining in her lower jaw. No subcutaneous adipose tissue was apparent and the breasts were atrophic. The uterus and ovaries showed marked atrophy. There was unusual prominence and distention of the peripheral veins.

The blood examinations obtained on her several admissions since 1944 have shown a slight anemia. The hemoglobin concentrations ranged from 11.8 to 14 Gm. per 100 ml. The erythrocyte counts varied from 3.4 to 4.0 million per cu. mm., and the leucocytes

from 5000 to 8500 per cu. mm. Routine urinalyses were normal. Serologic tests for syphilis gave negative reactions. The basal metabolic rates were slightly decreased without any corresponding increase in the concentration of serum cholesterol. For example, on one occasion when the basal metabolic rate was minus 19 per cent (the lowest measurement obtained), the serum cholesterol was 194 mg. per 100 ml.

X-ray examinations of the skull, pituitary fossa, sinuses, chest, gallbladder, and entire gastro-intestinal tract showed nothing abnormal. Water excretion tests for adrenal cortical function by the method of Robinson, Power and Kepler (2) were normal.

Ballistocardiographic studies by Dr. Isaac Starr showed an excessive circulation rating of plus 43 per cent estimated in terms of her actual weight but only plus 9 per cent when estimated in terms of her ideal weight.

It should be mentioned that the patient did not exhibit clinical evidence of avitaminosis at any time.

RESULTS

Our methods of analyses are given in a previous paper (1). The distribution of the serum components and comparisons with the fasted individual and the normal range of values are given in Table 1. It will be seen that in both the patient with anorexia nervosa and the fasted individual the concentrations of serum chloride were remarkably decreased. In our patient with anorexia nervosa, values of serum chloride between 50 and 60 mEq. per liter have practically always been obtained; in the fasted subject the concentration of serum chloride on the 45th day of fasting was 74 mEq.

TABLE 1. SERUM ANALYSES

	F.J. (Anorexia Nervosa)	B.D. (Prolonged Fasting—45 days)	Normal
Cl	58	74.0	99–104 mEq./L.
CO_2	138	100	55–60 vol. per cent
Total Base	129	141	143–148 mEq./L.
K	3.0	—	3–4 mEq./L.
Ca	10.6	12.2	9–11 mg./100 ml.
Mg	2.8	2.7	1.9–2.0 mEq./L.
P (Inorg.)	3.7	3.4	3–4 mg./100 ml.
Protein	6.3	6.2	6–7 Gm./100 ml.
Albumin	4.6	5.0	3.3–4.3 Gm./100 ml.
Globulin	1.7	1.2	2.2–2.8 Gm./100 ml.
Cholesterol	245	198	170–190 mg./100 ml.
Ester. Chol.	200	184	90–114 mg./100 ml.
Urea Nitrogen	9	24	9–17 mg./100 ml.
Uric Acid	5.3	9.0	3–4 mg./100 ml.
Creatinine	1.6	1.4	1–1.2 mg./100 ml.
Glucose	62	63	80–110 mg./100 ml.

per liter. Although our patient had vomited after meals on a number of occasions and especially when chlorides were administered intravenously, nevertheless, it would seem to us that the decreased concentration of serum chloride cannot be explained entirely on this basis. Moreover, a similar decrease in chloride was observed in the fasted individual who had not vomited during his fasting period.

It will be seen that the decreased concentration of serum chloride was compensated by an increase in the concentration of bicarbonate. Although this inverse relationship is unexplained, it seems reasonable to relate it to diminished intake of chloride with substitution of bicarbonate derived from endogenous sources. The decreased concentration of total base was due to a decrease in the concentration of serum sodium. Similar reduction in serum sodium concentration has been reported in Simmonds' disease

TABLE 2. ELECTROPHORETIC ANALYSIS OF SERUM, (F.J.), ANOREXIA NERVOSA

Total Protein	7.09 Gm. per 100 ml.
Albumin	4.50 Gm. per 100 ml.
Total Globulin	2.59 Gm. per 100 ml.
Alpha Globulin	0.49 Gm. per 100 ml.
Beta Globulin	0.92 Gm. per 100 ml.
Gamma Globulin	1.18 Gm. per 100 ml.

(3). It should be noted that the diminutions in the concentrations of serum chloride both in the patient with anorexia nervosa and in the fasted individual were much greater than the diminutions in serum total base. From these studies it may be inferred that the factors regulating the serum electrolytes are more concerned with the maintenance of a normal distribution of cations than of anions.

The increase in the magnesium concentration in the serum of both individuals has been of particular interest. Hypermagnesemia is reported as a characteristic finding in animals during hibernation (4) and has recently been reported (5) in an infant with a hypothalamic lesion having subnormal temperature and a depressed metabolic state.

The concentrations of the total serum protein were normal both in the patient with anorexia nervosa and in the fasted subject. It will be observed however, that upon salting out the globulins, the albumin fractions in both individuals were greater than normal, thus yielding exceptionally high albumin-globulin ratios.

In Table 2 is given the electrophoretic analysis of a sample of serum.

It should be noted that in this sample the concentrations of the four components were essentially within the normal range of values.

The total cholesterol in the serum reported in Table 1 was 245 mg. per 100 ml. and the esters were over 80 per cent of the total. Since both serum albumin and cholesterol esters are probably synthesized by the liver, the increased levels of these components suggest that there was an unusual degree of hepatic activity during which these components were liberated. That this seems reasonable is suggested by the studies of Addis, Poo and Lew (6) who found that the livers of rats may lose as much as 40 per cent of their original protein after a seven day fast.

Of the nonprotein nitrogen components the uric acid and creatinine

TABLE 3. FLUID DISTRIBUTION

	F.J. Anorexia Nervosa	B.D. Prolonged Fasting	Normal
Serum Volume	ml.	ml.	ml.
ml./Kg. present weight	68.0	57.7	43–48
ml./Kg. initial weight	38.9	37.8	
Total serum volume	1856	2539	
Extracellular Water	Per cent	Per cent	Per cent
(present weight)	17.6	33.4	20–25
(initial weight)	10.1	23.6	

concentrations were increased in both individuals. The concentration of urea nitrogen was increased in the fasted subject but not in the patient with anorexia nervosa. Increase in the concentrations of the nonprotein nitrogen components has been reported by a number of investigators during fasting (7, 8, 9) and would appear to be due to the increased catabolism of protein. The normal concentrations of urea nitrogen observed in anorexia nervosa may be due to minimal catabolism of protein. The concentrations of serum glucose during fasting were decreased below the normal range of values in both individuals.

In Table 3 are given the values for the serum volume and the volume of extracellular fluid (thiocyanate space). The values for serum volume in our patient with anorexia nervosa as well as in the fasted subject were greatly increased when calculated in relation to actual body weight. However, when calculated in relation to the initial or ideal weight, the values for serum volume were decreased below the normal range. The prominence and distention of the superficial veins, as well as the ballistocardiographic studies previously mentioned, suggest that the volume of the circulatory

bed had not been greatly altered. In this connection it might be mentioned that during starvation the heart and brain lose percentilely less weight than other organs (10).

The difference in the volume of extracellular fluid between the patient suffering from anorexia nervosa and the fasted subject was striking. In the fasted subject the extracellular fluid volume was elevated in relation to his fasting weight but when calculated in relation to his prefasting weight it was within the normal range of values. On the other hand, the patient suffering from anorexia nervosa revealed a striking percentile decrease in the extracellular fluid volume in relation to her actual weight and obviously in relation to her ideal weight. It seems possible, therefore, that this dissimilarity may be due to the differences in the lengths of time during which these conditions lasted.

TABLE 4. ASSAYS OF URINARY HORMONES
(24-hour excretion)

	F.J. Anorexia Nervosa	Normal
Gonadotropins	none	8– 30 m.u.
Estrogens	<6 m.u.	22–100 m.u.
Neutral 17-Ketosteroids	2.3 mg.	9– 15 mg.

Assays of the urinary hormones in the patient with anorexia nervosa are given in Table 4. As might have been anticipated the excretion of gonadotropins was minimal,—in fact none was detected in 24-hour samples of urine. The excretion of estrogens and neutral 17-ketosteroids was very low.

In Table 5 are given the results of the insulin, galactose and glucose tolerance tests in our patient. Fraser and his colleagues (11) have suggested the use of the insulin sensitivity test in the differential diagnosis between pituitary cachexia (Simmonds' disease) and anorexia nervosa. According to these investigators, in normal individuals and in patients with anorexia nervosa the concentrations of fasting blood sugar are within the normal range of values; one-half hour after giving intravenously 0.1 unit of insulin per kilogram of body weight, the concentration of blood sugar is decreased, returning to the normal fasting level within two hours. On the other hand in pituitary cachexia, the concentration of blood sugar is decreased at the end of one-half hour and remains decreased until after two hours. On three occasions in which this test was tried in our patient, the concentrations of blood sugar were decreased below the normal range at the one-half hour

period and remained so for two hours. In spite of these positive insulin tolerance tests we believe that our patient was probably suffering from anorexia nervosa rather than from an organic lesion of the anterior pituitary gland (Simmonds' disease). However, the differentiation between anorexia nervosa and Simmonds' disease is an extraordinarily complex one. Whether minor destructive lesions occur in the pituitary gland in anorexia nervosa and indeed whether the two diseases are independent of each other is speculative at the present time. In Table 5 it will be observed that in one

TABLE 5. TOLERANCE TESTS, (F.J.), ANOREXIA NERVOSA

	Blood Sugar mg./100 ml.		
Insulin Tolerance Test	7/13	7/19	11/27
Fasting	58	69	66
5 minutes	66	—	—
30 minutes	44	40	16*
1 hour	—	12*	32
2 hours	—	46	57
Glucose Tolerance Test (2-dose)			
Fasting	62	83	
30 minutes	128	95	
1 hour	119	92	
Galactose Tolerance Test		Blood Galactose (mg. per 100 ml.)	
5 minutes	11		
30 minutes	15		
1 hour	4		

* Symptoms of shock.

of the glucose tolerance tests the concentration of blood sugar did not rise to the usual level observed in normal individuals. In this connection it should be noted that although the concentrations of the fasting blood sugar have been frequently decreased in our patient, at no time did she manifest symptoms of spontaneous hypoglycemia. In anorexia nervosa, in contrast to Simmonds' disease, spontaneous hypoglycemia has not been reported.

The galactose tolerance test according to the method of Althausen (12) was essentially normal.

CHLORIDE BALANCE

Of particular interest was the finding that our patient continued to excrete chloride in the urine even when the concentration of serum chloride

TABLE 6. CHLORIDE BALANCE AND SERUM ANALYSES

Dates	Intake Gm. NaCl			Output Gm. NaCl			*Balance Gm. NaCl	Cl mEq./L	CO_2 Vol. per 100 ml.	Total Protein Gm. per 100 ml.	Total Base mEq./L	Cl^- mEq./l.	HCO_3^- nM/L	Pr^- nM/L	Measured Anions mEq./L	Remarks
	Diet	Intravenous	Total	Urine	Vomitus	Total										
1/27	3.25 (55.6)*	10.81 (184.9)	14.06 (240.6)	0.19 (3.3)	7.63 (130.5)	7.82 (133.8)	+6.24 (+106.8)	64.0	119	6.4	123.8	64.0	51.8	13.0	128.8	
1/28	3.10 (53.0)	13.74 (235.1)	16.84 (288.1)	0.66 (11.3)	8.59 (147.0)	9.25 (158.3)	+7.59 (+129.8)	94.0	85	4.6	136.0	94.0	26.7	9.3	130.0	
1/29	3.80 (65.0)	12.91 (220.9)	16.71 (285.9)	1.00 (17.1)	10.86 (185.8)	11.86 (202.9)	+4.85 (+83.0)	92.1	82	4.4	136.0	92.1	25.3	8.9	126.3	Discontinued on account of edema
1/30	4.34 (74.3)	11.23 (192.1)	15.57 (266.4)	0.86 (14.7)	16.13 (276.0)	16.99 (290.7)	−1.42 (−24.3)									
1/31	4.60 (78.7)	4.35 (74.4)	8.95 (153.1)	0.87 (14.9)	11.63 (199.0)	12.50 (213.9)	−3.55 (−60.8)									

* Values in parentheses represent chloride expressed as mEq.

was between 50 and 60 mEq. per liter. The amounts of chloride excreted were small, to be sure; however, they were readily measurable and ranged from 0.25 to 0.5 grams of NaCl (4.3 to 8.6 mEq. chloride) per day. Normally from 6 to 12 grams of chloride as NaCl (100 to 200 mEq. chloride) are excreted in the urine in 24 hours. Chloride usually disappears from the urine, except for traces, when the serum chloride falls to levels below 80 mEq. per liter. Although the so-called renal threshold for chloride is probably not as definite a level as was once believed, nevertheless, there is undoubtedly a normal tendency for the body to conserve chloride and to prevent its excretion in the urine when the chloride concentration in the serum is decreased below the normal range of 98 to 104 mEq. per liter. It would appear that the threshold for excreting chloride in our patient with anorexia nervosa has been remarkably reduced. Although the water excretion test for adrenal function was never positive, the urinary excretion of neutral 17-ketosteroids was low. This suggests some functional insufficiency of her adrenal cortex. Such insufficiency may possibly have reduced the ability of the renal tubules to reabsorb chloride and thus may have contributed to the reduced levels of serum total base and chloride.

In Table 6 are given data pertaining to the measurements of chloride balance and concentration of serum components undertaken at a time when the patient was given NaCl intravenously. Soon after commencing saline infusions, the patient began to vomit. It will be seen that during the five day period of this therapy, the patient showed a positive chloride balance from 4.85 to 7.59 grams of NaCl (83 to 130 mEq. chloride) per day during the first three days. Since the patient was constipated, the amount of Cl excreted in the stool, which is presumably less than 0.5 grams NaCl (8.6 mEq. chloride) per day, was not included. At the time of these studies the concentration of serum chloride increased from 64.0 to 94.0 mEq. per liter and the total serum CO_2 decreased from 119 to 85 volumes per cent. The concentration of total serum protein also decreased to 4.6 grams per 100 ml. During the next two days vomiting became increasingly severe and the vomitus contained increasing amounts of chloride. Moreover, the patient became strikingly edematous. The intravenous administration of NaCl was therefore discontinued. Within two weeks thereafter, the patient lost her edema, and her serum electrolytes reverted to their previous pattern.

COMMENT

The pattern of our patient's personality conformed closely to that seen typically in anorexia nervosa. Moreover, we were unable to discover or to eradicate the basic psychic abnormality which was almost certainly the cause of her initial compulsion to restrict her diet at a time when she weighed

only 112 pounds. The gradual replacement of a reduced caloric intake by prolonged periods of fairly normal diet accompanied by postprandial vomiting emphasizes the ineptness of the term "anorexia nervosa." Anorexia in the true sense of the term is by no means always seen, even when undernutrition results from reduced food intake rather than from habitual vomiting. The dietary restriction often results more from the exercise of will than from actual distaste for food. Likewise, the term "nervosa" is meaningless. The substitution of a more accurately descriptive term, such as "chronic psychogenic undernutrition," appears desirable. However, the term "anorexia nervosa," like many other vague and inaccurate descriptive names in medicine, has become so deeply rooted by custom that its elimination seems well nigh impossible.

Our patient showed the usual disparity between the degree of cachexia and the extent of actual muscular asthenia. Controversy has long existed concerning the relative importance of psychic factors versus physiologic derangement of certain organ systems, especially the endocrine, in some chronically undernourished patients. This difficulty has resulted in considerable confusion of terminology in the literature, particularly in regard to the diagnosis of pituitary cachexia. Many cases are described as examples of anorexia nervosa. The evidence in our case points toward the development of physiologic depression of the endocrine system probaby as a result of chronic inanition which in turn was due to primary psychic or emotional factors. In this sense, then, the existence of a true functional hypopituitarism in the later stages of the disorder must be admitted, and such a functional hypopituitarism probably contributes in large part to the state of depression found in the other endocrine target-organs (thyroid, adrenals, gonads). Nevertheless, there remain in most cases both objective and subjective distinctions which usually make it possible to differentiate between the functional and the truly organic types of anterior pituitary deficiency. It, therefore, appears desirable to reserve the term "pituitary cachexia" (Simmonds' disease, panhypopituitarism, cachexia hypophyseopriva) for those cases in which organic destructive lesions of the anterior pituitary (including atrophy with fibrosis) can be demonstrated or reasonably inferred.

SUMMARY

Studies of the changes in composition and distribution of the body fluids and serum electrolytes have been made in a patient with anorexia nervosa. These have been compared with similar studies previously reported in a severely emaciated individual who had undergone a voluntary fast for a period of 45 days.

The concentrations of serum chloride were remarkably decreased in both

individuals. The decreased concentrations of chloride were compensated by increases in the bicarbonate. Although the concentrations of serum total base were decreased in both subjects, the decreases of total base were much less than those of chloride. It appears that the factors regulating the serum electrolytes are more concerned with maintenance of normal distribution of cations than of anions.

The concentrations of serum magnesium were increased in both individuals.

The concentration of serum albumin, cholesterol esters, uric acid and creatinine were increased in both subjects.

The serum volume in each subject was increased when calculated in relation to actual weight, but was decreased when estimated in relation to the normal weight.

The volume of extracellular fluid was decreased in the patient with anorexia nervosa. In the fasted subject the volume of extracellular fluid was increased in relation to his actual weight, but was within the normal range when calculated in relation to his prefasting weight.

The cardiac output (ballistocardiographic method) was increased in each subject when estimated in relation to actual weight, but was normal when estimated in relation to ideal weight.

The patient with anorexia nervosa continued to excrete chloride in her urine even when the concentration of serum chloride was below 60 mEq. per liter.

Measurements of chloride balance made during a period of intravenous administration of NaCl in the patient with anorexia nervosa showed a positive chloride balance for the first three days; during this period the concentration of serum chloride increased from 64.0 to 94.0 mEq. per liter.

The patient with anorexia nervosa showed increased sensitivity to insulin, lowered basal metabolic rates and decreased urinary excretion of estrogens, gonadotropins and neutral 17-ketosteroids. These findings suggest functional insufficiency of the anterior pituitary, gonads, and perhaps of the thyroid and adrenal cortex. The relationship between the syndrome of anorexia nervosa and functional insufficiency of the endocrine system has been briefly discussed.

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A DIURNAL RHYTHM IN THE EXCRETION OF NEUTRAL REDUCING LIPIDS BY MAN AND ITS RELATION TO THE 17-KETOSTEROID RHYTHM*

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THE observation that there is a diurnal rhythm in urinary ketosteroid excretion (1, 2, 3) and that this may be related to stress-stimulated adrenocortical secretion (2) led us to examine other possible indices of adrenocortical secretion. The development of a method for measuring neutral reducing lipid in urine by Heard and Sobel (4) and the indications that this material represents in large part corticosteroid possessing reducing activity by virtue of its α-ketol sidechain (4, 5, 6), offered an opportunity to assess the diurnal changes in excretion of adrenocortical substances, which are entirely different from those of the 17-ketosteroids.

MATERIAL AND METHODS

Twenty-four normal healthy men ranging in age from 24 to 72 years (see Table 1) were the subjects of this investigation. Urine was collected over a 24-hour period in three lots covering approximately the periods 11 p.m. to 7 a.m. (sleep), 7 a.m. to 11 a.m. (morning) and 11 a.m. to 11 p.m. (day).

From an aliquot of each collection, fractionation for the neutral ketones and 17-ketosteroid determination was made by methods previously described (7). A second aliquot was employed for determining neutral reducing lipid by the Heard-Sobel method (4). In our experience the urine of normal men ordinarily contains sufficient 17-ketosteroid and neutral reducing lipid to make the equivalent of a half-hour collection sufficient for accurate colorimetric determination. Creatinine determinations were made routinely on each specimen (7).

RESULTS

In Table 1 we present for each subject the urinary outputs of neutral reducing lipid and 17-ketosteroid respectively, on the basis of mg. per

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twenty-four hours and mg. per Gm. of creatinine. An inspection of the table indicates that for both types of steroid and on either measurement basis, the great majority of subjects have minimal outputs during the sleep period. Actually the 17-ketosteroid measurements on a 24-hour basis show only two men in whom the sleep value is not less than either of the waking values; on a gram-creatinine basis there are three such men. Similarly the sleep "cortin" output is highest in only four subjects on the 24-hour basis and in only two on a gram-creatinine basis.

TABLE 1. NEUTRAL REDUCING LIPID* ("CORTIN") AND 17-KETOSTEROID URINARY OUTPUTS OF NORMAL, HEALTHY MEN

Subject No.	Age Yrs.	Mg. per 24 Hours						Mg. per Gm. of Creatinine					
		"Cortin"			17-Ketosteroids			"Cortin"			17-Ketosteroids		
		Sleep	Morning	Day	Sleep	Morning	Day	Sleep	Morning	Day	Sleep	Morning	Day
1	24	1.06	2.58	1.74	6.24	18.72	9.36	0.56	1.26	0.72	3.27	9.15	3.88
2	24	2.42	2.10	2.13	4.32	12.24	16.56	1.01	0.69	1.02	1.80	4.01	7.92
3	24	1.39	2.91	1.71	14.16	29.76	16.32	1.00	1.29	1.14	10.20	13.20	10.90
4	26	0.71	0.77	1.46	11.28	6.72	14.16	0.38	0.39	0.80	5.96	3.44	7.74
5	27	0.62	2.32	1.51	9.60	12.72	7.92	0.57	1.69	1.18	8.90	9.30	6.18
6	27	1.56	1.83	3.23	14.16	16.32	15.84	1.00	1.33	1.63	9.08	11.85	8.00
7	27	2.03	2.34	1.02	12.96	19.68	19.20	1.48	1.18	0.55	9.45	9.93	10.40
8	28	0.58	1.71	1.39	8.88	4.80	10.56	0.38	1.20	0.70	5.75	3.36	5.28
9	29	0.98	2.42	1.49	11.28	20.16	8.88	0.54	1.21	1.05	6.27	10.08	6.25
10	30	1.17	2.40	1.75	13.20	24.24	21.36	0.54	1.35	0.98	6.63	13.60	12.20
11	32	3.39	3.25	3.28	10.56	16.80	12.24	2.13	2.34	2.26	6.65	12.10	8.45
12	33	1.51	2.30	1.48	21.60	15.84	27.36	0.99	1.76	0.61	14.20	12.10	11.30
13	34	0.63	1.14	0.69	4.80	10.08	7.92	0.42	0.60	0.37	3.20	5.40	4.25
14	34	0.82	1.70	1.16	7.44	9.60	9.60	0.49	1.15	0.72	4.48	6.50	5.93
15	35	1.14	2.49	1.56	3.12	11.04	4.32	1.07	1.25	0.97	2.92	5.55	2.03
16	35	0.55	1.22	1.34	8.40	13.92	7.44	0.31	0.61	0.60	4.80	6.96	3.30
17	35	1.65	2.10	1.42	9.60	14.16	6.48	0.88	1.36	0.98	5.10	9.20	4.38
18	37	2.05	1.83	2.02	7.68	7.44	7.20	1.46	2.35	1.85	5.50	9.55	6.60
19	39	1.03	2.18	1.08	12.48	17.04	15.60	0.55	1.04	0.45	6.70	8.10	6.50
20	39	1.59	2.43	1.95	7.92	11.28	12.00	0.78	1.45	0.78	3.90	6.70	4.67
21	44	2.30	3.04	2.56	6.96	24.24	19.68	1.98	2.08	1.42	6.00	16.60	10.90
22	47	2.39	2.83	1.86	2.16	6.96	5.76	1.59	1.90	0.96	1.44	4.67	2.99
23	56	2.37	1.70	2.11	5.52	3.12	4.08	1.31	1.09	1.06	3.05	2.00	2.04
24	72	0.80	1.23	1.35	1.68	3.60	3.12	0.78	0.95	0.88	1.65	2.77	2.04

* Expressed as 11-desoxycorticosterone equivalent.

We have previously reported a general pattern of daily excretion of 17-ketosteroid involving a maximum output shortly after waking and a decline thereafter to minimal values at night (1, 2, 3). Departures from this pattern are occasioned by stressful activity (2, 3, 6) but where it generally obtains, we would expect "sleep" outputs to be low, the "morning" output highest and the "day" values less than the "morning." Among these 24 sets of collections, 19 exhibit this typical pattern on the hourly calculation, 18 on the gram-creatinine basis. The data of Table 1 show a similar excretion pattern for the neutral reducing lipid; 17 and 19 sets of determinations

show this pattern among the 24-hour rate and gram-creatinine values respectively.

In Table 2 we present the mean output values for the 24 subjects and the percentage increase of the morning and day values over the sleep values. We previously reported approximately a 60 per cent increase of morning over sleep values (5) of 17-ketosteroid calculated on an hourly excretion basis. For these data the increase is 58.1 per cent. The neutral reducing lipids show a mean increase of 47.3 per cent for the same period. The

TABLE 2. THE MEAN URINARY OUTPUT VALUES FOR 17-KETOSTEROID AND NEUTRAL REDUCING LIPID AND THE VARIABILITY THEREOF

	Sleep	Morning		Day		Values of "t" for per cent difference between sleep and		Coefficients of Variation (%)		
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
	Mg.	Mg.	Per cent increase over sleep	Mg.	Per cent increase over sleep	Morning	Day	Sleep	Morning	Day
Neutral reducing lipid—per 24 hrs.	1.45 ±0.153	2.12 ±0.128	47.3	1.72 ±0.124	18.6	3.40	2.57	51.7	29.6	35.3
17-ketosteroid per 24 hrs.	9.00 ±0.929	13.77 ±1.404	58.1	11.79 ±1.259	31.0	3.14	2.66	50.6	50.0	52.3
Neutral reducing lipid—per Gm. creatinine	0.93 ±0.104	1.31 ±0.104	40.8	0.97 ±0.091	6.4	3.33	1.96	54.8	39.0	44.8
17-ketosteroid per Gm. creatinine	5.70 ±0.637	8.17 ±0.792	43.3	6.45 ±0.629	13.21	3.21	1.34	54.7	47.5	47.8

percentage differences between the sleep and morning values for both 17-ketosteroid and "cortin" are statistically significant on either basis of calculation ($p < 0.01$, column 6). The percentage differences between sleep and day values lie just below the 2 per cent level of confidence ($p = 0.02$ when $t = 2.5$ @ 23 degrees of freedom) when the mg. per 24-hour values are examined; but lie above the 5 per cent level of confidence on a gram-creatinine basis. The data then, on the whole, exhibit significant rises in output for the waking hours and demonstrate a genuine diurnal change. The percentage increase in 17-ketosteroid values for the waking hours is, on the average, higher than the corresponding "cortin" increases (columns 3 and 5) but in these data the differences are not statistically significant. This may in part be due to the rather large variation between men. We have previously observed that each individual tends to excrete 17-ketosteroid at

a fairly characteristic level but that among normal men there is a wide absolute scatter (1, 2). The coefficients of variation listed in Table 2 (columns 8, 9, 10) illustrate this variability. It is interesting that in the morning values and to a lesser extent in the day values, the scatter is decreased for the "cortin" but not particularly for the 17-ketosteroid (cf. columns 9 and 10 with column 8).

In view of the fact that the outputs of both 17-ketosteroid and neutral reducing lipid show the same sort of diurnal rhythm, we have attempted to see if they are correlated. In Table 3 we present the correlation coefficients for the absolute outputs and the percentage and absolute increases. These coefficients were calculated on the mg. per 24-hour output data. The only

TABLE 3. CORRELATION COEFFICIENTS FOR VARIOUS 17-KETOSTEROID AND NEUTRAL REDUCING LIPID DATA

	Period	1. Sleep*	2. Morning*	3. Day*	4. All Values†
A	Reducing lipid vs. 17-ketosteroid absolute outputs	+0.046	+0.612	+0.153	+0.372
	Period	**Per cent change, morning over sleep***	**Per cent change, day over sleep***	**Absolute change in output, morning over sleep***	**Absolute change in output, day over sleep***
B	Reducing lipid vs. 17-ketosteroid output changes	+0.024	−0.319	+0.377	−0.214

* D.F. = 23 r @ 5% level of confidence = 0.396
† D.F. = 70 r @ 5% level of confidence = 0.232
r @ 1% level of confidence = 0.302

significant correlation coefficients are those for the absolute output levels for all the data (column A4) and especially for the morning outputs (column A2). It is clear that the significant overall correlation is heavily weighted by the high correlation for the morning outputs. These significant positive correlations indicate that in these men, a relatively high output of 17-ketosteroid is generally accompanied by a high output of "cortin" and vice versa; and that this is especially true in the morning hours. Since this is the period when the values of both 17-ketosteroid and "cortin" tend to be at a maximum, we might expect a significant correlation between the absolute increases of each over the sleep level (column B3). The correlation coefficient of +0.377 actually obtained just fails of significance. Since

no significant correlation for output changes is obtained either on a percentage or absolute basis we can only conclude that the factors causing 17-ketosteroid to change are not necessarily the same which cause the neutral reducing lipid to change. Calculation of possible correlation between output changes on the basis of gram-creatinine output gives similar nonsignificant values.

DISCUSSION

These data clearly indicate significant diurnal changes in both 17-ketosteroid and neutral reducing lipid output. Moreover the diurnal pattern of urinary excretion is similar for each. Nonetheless, the lack of significant correlation between the changes in the two outputs implies independent variation in these changes. We have previously shown (6) that psychomotor stress in normal men evokes an increased excretion of both 17-ketosteroid and "cortin," whereas the administration of glucose evokes an increased output of the latter but not the former. Since the specimens taken during the waking hours include mealtimes, the carbohydrate ingested (or formed from the food) may especially influence the "cortin" produced. The implication that the adrenal cortex may secrete 17-ketosteroid precursors independent of neutral reducing lipid precursors is most interesting. The fact that there is significant positive correlation between all absolute output values (Table 3, A4) implies that the general level of secretion of these precursors goes hand in hand. It is puzzling, therefore, that there is no significant correlation for the absolute sleep values (Table 3, A1) when one is presumably dealing with a basal level of adrenocortical activity. While it is recognized that 17-ketosteroid precursors may be secreted by the testis, they presumably contribute only a fraction of the urinary output and there is no evidence that testis secretion varies diurnally.

Further consideration of the lack of correlation between changes in these two indices of adrenocortical function leads to the consideration that in the human subject different adrenocorticotropins may be involved. The pituitary seems to be essential for an increase of adrenocortical secretion during the alarm reaction (8) and if we consider these diurnal changes as responses to the stresses of daily life a differential evocation of pituitary adrenocorticotropin is suggested. It is interesting to note that Hemphill and Reiss (9) in comparing reducing lipids in adrenals and blood after cold stress and corticotropin administration in animals, obtain different responses. That urinary 17-ketosteroid and corticoids do not go hand in hand has already been shown by Venning and Kazmin (10) for normal individuals and by Venning (11) for pregnant women. Venning's measurements were for glycogenic urinary steroids and these are certainly not identical with the neutral reducing lipids we have measured.

SUMMARY

The urinary output of 17-ketosteroid and neutral reducing lipid was measured simultaneously on aliquots of "sleep," "morning" and "day" samples from 24 normal, healthy men aged 24 to 72 years. The great majority of these subjects showed minimal excretion of both types of substance during the sleep period, maximal output in the morning and less than morning values in the day specimens, when the output is expressed on either a per hour or a per gram of creatinine basis. The mean output increases for both the morning and day excretions are statistically significant. There is a significant correlation between absolute levels of 17-ketosteroid and neutral reducing lipid. Although a similar diurnal pattern of excretion occurs for both types of urinary substance the changes in excretion (on either a percentage or absolute change basis) of each are not significantly correlated. This is taken to indicate the factors evoking urinary 17-ketosteroid increase (or decrease) are not the same as those evoking neutral reducing lipid change.

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EFFECTS OF STARVATION ON SEX HORMONES IN THE MALE

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I. INTRODUCTION

FOR many years there has been considerable interest in the relation of sex hormones to changes in the skin, sebaceous glands, hair, libido, prostatic fluid, breasts and menstruation. An immense amount of research has been completed relative to sex hormones, but much of this work has been carried out on small animals. The question has frequently arisen: "Would the results have been the same in the human?" Thirty-eight months in Japanese Prisoner of War Camps presented excellent opportunities to observe the clinical effects of starvation on the sex hormones of the human. The observations are set forth in this paper, and are compared to some of the results of experiments which have been carried out on small animals by various investigators.

II. CONDITIONS IN JAPANESE PRISON CAMPS

During the few months on Bataan and Corregidor, the slim diet of rice and fish had been cut to half-rations, and finally to quarter-rations. Many of the front-line troops went for days without food. When the Fil-American Forces surrendered during April and May of 1942, many vitamin deficiency diseases were already well established. Then came the "Death March" from Bataan to Camp O'Donnell without food, and with only the little water that could be sneaked from the ditches alongside the road, when the Japanese guards were not watching.

At Camp O'Donnell prisoners died at the rate of several hundred each day. The majority of deaths were among the Filipino troops, because of their proportionately larger numbers, not because of any difference in the treatment of the two groups.

In June 1942 the American prisoners were segregated and taken on a second "Death March" to Cabanatuan, Nueva Ecija. No preparation had been made at the new camp for the reception of the exhausted and starved prisoners. They were crowded into grass shacks, where each man was allotted two by six feet of floor space for sleeping. Many were without blankets and mosquito-nets, and very few had adequate clothing or shoes. Diseases (malaria, dysenteries, vitamin-deficiency diseases and diphtheria)

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were prevalent. Sanitary provisions were completely wanting. Medical care was also lacking, because the doctors and medical corpsmen were as exhausted, starved and sick as the other prisoners, and there was very little medicine.

After a few months in the new camp, all of the prisoners who were still able to walk were forced to work all day in the hot sun on the "Farm."

III. STARVATION DIET

The diet consisted of two to four hundred grams of a very poor grade of dirty rice, and greens (the weeds of carabao wallows). About once a week or less a carabao (water buffalo) was killed for meat. The Japanese took the choice parts, and donated the remains to the American "Mess Shacks" to be cooked into a thin gravy for the nine thousand prisoners. The few prisoners who had been able to smuggle money into camp, and those who got paid for working on the "Farm," were able to purchase bananas, mango beans, camotes (sweet potatoes), and duck eggs in very small quantities, and at very inflated prices. When the pump was working, one canteen of water could be obtained by standing in line for an hour or more.

IV. MORTALITY AND MORBIDITY

The mortality and morbidity were both high. As many as fifty and occasionally more, shrivelled and bloated bodies were daily dropped into common graves. The Japanese were repeatedly asked for medicines, vitamins and additional food, but always gave the same answer: "Mati mati" meaning "After a while."

By December 1942 (eight months in prison camp) more than twenty-four hundred prisoners had died at Cabanatuan Prison Camp. Another twenty-five hundred lay seriously ill on the floors of the makeshift hospital. Most of these patients had lost as much as fifty per cent of their body weight. All of the diseases described in the literature as associated with vitamin deficiency had become prevalent.

V. EVIDENCE OF ANDROGEN SUPPRESSION

Early in starvation there was a universal loss of libido, and an absence of nocturnal emissions. Several months later many of the prisoners complained that their hair was becoming thin. The hair on the head and face became soft, fine and sparse. Where it had been necessary to shave daily prior to imprisonment, shaving once a week was now sufficient. Hairy chests, arms and legs became almost bare. Axillary and pubic hair became thin, with a tendency in some for the hair to assume the feminine distribution.

The skin became thin and loose. It could be picked up from the under-

lying tissue and freely moved about. The sebaceous glands of the face, chest and back became atrophic, and no longer contained comedos. The oiliness of the skin disappeared. Prisoners who had previously been affected with acne vulgaris and seborrheic dermatitis were freed of these conditions.

Some claimed that their testes had atrophied, but this could not be accurately checked, as no measurements had been taken prior to starvation.

VI. EVIDENCE OF ANDROGEN STIMULATION

In December 1943 and the months following, each prisoner received four parcels (eleven pounds each) of Red Cross food and vitamins. The extra food and vitamins made the diet adequate for a period of from two to four months, depending upon the individual and his ability to eat sparingly. The death rate dropped suddenly from three hundred a month to two or three a month. The general health of the camp improved. There were weight gains of from five to twenty pounds.

It was only a matter of several weeks after the diet became normal, that libidos, erections, and nocturnal emissions returned. A few weeks later, prisoners noted hair returning to the bare areas, beards becoming thicker and tougher, sebaceous glands becoming active, and in a few cases the reappearance of acne vulgaris and seborrheic dermatitis.

VII. "TRUE" GYNECOMASTIA

From three to twelve weeks after the diet became adequate, gynecomastia appeared in 300 (six per cent) of the fifty-five hundred prisoners remaining in camp. The ages varied from 18 to 64 years. Fifteen per cent gave a previous history of gynecomastia at puberty. At least fifty per cent were bilateral, although it was usual for one tumor to precede the second by from one to eight weeks. The size varied from 2 to 5 centimeters (average 24 millimeters). Ninety per cent were tender or painful. Three per cent secreted a colostrum-like substance. In only one case did it have the appearance of milk. No definite history of injury was obtained in any of this series. The tumors reached maximum size in from one to nine weeks, and remained stationary until they began to disappear spontaneously (average, four months). None showed any tendency to grow after the size became stabilized. None became malignant during the period of observation.

VIII. FURTHER STARVATION

The Red Cross food and vitamins were largely consumed within two to four months. The diet reverted to rice and greens, supplemented by a rare duck egg, banana, or small portion of mango beans. The diet was

again definitely inadequate, especially in fat and protein. Deficiency diseases and the "castration syndrome" gradually reappeared. Gynecomastias slowly became painless, and disappeared over a period of from one to twenty-four months (average four months).

IX. LIBERATION—ADEQUATE DIET

Finally after twenty more months of starvation and disease, American food arrived via parachutes just prior to liberation (August and September, 1945). The diet was adequate again. Prisoners gorged themselves. Weight gains of seven to fifteen pounds a week were the rule. Within a few weeks there was evidence that the hormones were being stimulated. A common greeting of the morning became "I'm a man again." Also within a few weeks gynecomastia made its reappearance. It was much more prevalent than it had been after the Red Cross parcels in 1943–44.

Hair gradually became thicker, and in some cases returned to bald areas. Sebaceous glands became hypertrophic and the skin oily. Acne vulgaris and seborrheic dermatitis returned to those previously affected. Thin and fragile fingernails and toenails became thicker.

Many of the prisoners held genuine fears of sterility and impotence, after more than forty months of starvation. However a lapse of two years since liberation has resulted in at least a normal number of pregnancies of the prisoners' wives, and in the normal births of apparently healthy babies.

X. DISCUSSION

It must be obvious to the reader that studies of the androgen-estrogen ratios and levels, and other laboratory determinations were impossible, as there were no facilities. However, an effort has been made to correlate the clinical observation made of the prisoners, to recent investigations of the effects of starvation, which have been made largely upon animals.

It has been known for some time that spermatic function of the testes is adversely affected by deficiency of vitamin B_1 or E, and that atrophy of the testes has resulted in some cases.

Pazos and Huggins (1) have shown in animals that complete deprivation of food from four to eleven days causes a cessation of prostatic fluid due to androgenic deficiency. They believe this is due to a decreased gonadotropin production, and found that they could restore the fluid by injections of testosterone propionate. In their experiments the testes remained capable of androgen production for about twenty-one days of starvation.

The symptoms seen in the starving prisoners, described in Section V, are similar to those seen in the castrated male (2), and are likewise attributed to hypogonadism and androgen suppression.

Hooker and Pffeiffer (3) have produced the "castration syndrome" (atrophy of hair follicles, loss of hair, thinning and loosening of the skin, and atrophy of the sebaceous glands) by treating animals with estrogen. They could protect the animal, however, from the castration syndrome by giving androgen as well as estrogen. It is very probable that the "castration syndrome" seen in the prisoners may have been produced by a relatively increased estrogen as well as a suppression of androgen. The recovery from the castration syndrome following the return of the diet to normal, is believed to be due to the stimulation of androgenic function. Thompson and Heckel (4) demonstrated similar results in the eunuch with large doses of testosterone (gradual increase of hair growth, erections, seminal emissions, and acne).

The three hundred cases of gynecomastia in this series developed after the diet became adequate, and not during starvation.

It has been rather well established that the male mammary duct system is sensitive to excess estrogen (5, 6). It responds with hyperplasia of the rudimentary ducts and periductal connective tissue (gynecomastia). Androgen and estrogen levels are normally maintained by the ability of the liver to inactivate excess (7). However, prolonged starvation impairs the liver (8, 9, 10) so that it cannot inactivate excess estrogen. It then appears that the gynecomastia following starvation is a result of excess estrogen subsequent to liver impairment (11, 12, 13). As the impaired liver gradually recovers its function of inactivating excess estrogen, the androgen-estrogen ratio returns to normal, and the gynecomastia gradually disappears.

SUMMARY

1. The effects of starvation on the sex hormones of the human appear to be the same as or similar to those of the animal.

2. Prolonged inanition produces symptoms (castration syndrome) indicating a decrease in sex hormone production.

3. Subsequent adequate diet causes a recovery from the syndrome, thus indicating a stimulation of the sex hormones.

4. Gynecomastia appeared in six per cent of the prisoners, when the diet became adequate subsequent to prolonged starvation, apparently due to imbalance of the sex hormones.

5. Gynecomastia following relief of starvation disappeared spontaneously, both during further starvation, and after several months of adequate diet.

6. Forty months of severe starvation did not produce the expected impotence or sterility in the recovered prisoners.

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THE RAPID RAT TEST FOR PREGNANCY

THE OVARIAN HYPEREMIA RESPONSE AS A ROUTINE DIAGNOSTIC PROCEDURE*

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SEVERAL authors have described rapid tests for pregnancy based on the hyperemic response of the immature rat ovary to pregnancy urine gonadotropin. Salmon and associates (1) observed that ovarian hyperemia was produced within six hours following a subcutaneous injection of pregnancy urine and suggested this as a test for pregnancy. Kupperman, Greenblatt and Noback (2) reported that the duration of the test could be reduced to two hours provided the urine was injected intraperitoneally.

The accuracy of the two-hour test (excluding observations in ectopic pregnancy) has recently been reported by Kupperman and Greenblatt (3) to be 99.5 per cent in a total of 752 tests. Bunde (4) using either the two-hour test with intraperitoneal injections or the six-hour test with subcutaneous injections, obtained an accuracy of only 84.5 per cent in 108 tests. A test developed by Zondek, Sulman and Black (5) consists of two subcutaneous injections of pregnancy urine at an interval of one hour. The accuracy of this test was 69 per cent at two hours, 92.2 per cent at six hours and 99 per cent at twenty-four hours.

The present report is a summary of the authors' experience with the hyperemia test when used as a routine laboratory test for pregnancy. In no instance was the clinical diagnosis known to the observer at the time the test was performed. The results of the tests have been compared with Aschheim-Zondek tests performed on the same urines. Further observations were made on the relationship of the duration of the test to the intensity of the hyperemic response and the variability in responsiveness of the test animals. An attempt has been made to identify those conditions which facilitate the performance of the test with greatest accuracy.

TECHNIQUE

The technique of Kupperman and Greenblatt (3) was followed in most details except that no attempt was made to restrict the duration of the test to exactly two hours.

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Test Animals. Immature female rats, weighing 35 to 75 Gm., are used for the test. Only occasionally has a rat in this range been found unsatisfactory for use due to sexual maturity. Immaturity is usually indicated by a lack of vaginal patency. Albino rats of the Wistar strain were used throughout this investigation.

Dosage and Mode of Administration. Two cc. of urine from a first morning sample are injected intraperitoneally into each of two animals. Since the diagnostic laboratory cannot be certain that such a specimen has been obtained, it is well to take the precaution of determining the specific gravity of the specimen. In the event of a particularly dilute urine, the dose may be increased by 1 or 2 cc.

The following method of holding the animal for injection has been found convenient. The animal is held in the left hand by grasping the loose skin at the back of the neck with the thumb and index finger and holding the tail against the palm with the little and ring fingers. Held thus, the animal is firmly restrained and the injections may be made with ease.

One cc. of urine is injected into the right and left lower abdominal quadrants. The animals are sacrificed by ether or chloroform asphyxiation two hours after the injections or at any later time up to twenty-four hours. In the present study approximately 85 per cent of the tests ran for four to six hours. The remaining 15 per cent of the tests were about equally divided between two-hour, three-hour and seven-hour tests.

Hyperemic Response. A positive hyperemic response is indicated by a reddened appearance of the ovary. This response is illustrated in Figure 1. In most instances in a positive test the heightened vascularity of the ovarian stroma and follicles causes the ovaries to stand out in striking contrast to the surrounding adipose tissue. In some cases, however, such a weak response may be obtained that only an experienced observer can read the test with any degree of accuracy.

Aids in Reading the Response. Observance of the following details has greatly assisted in reading the test. Light from the ordinary incandescent 60 or 100 watt bulb is better than sunlight or fluorescent light. The test animal, with ovaries exposed, is best held for observation 6 to 8 inches from the source of light and slightly to one side rather than directly beneath the bulb. At this position there is less tendency for generalized high-lighting of the exposed tissues.

To distinguish induced hyperemia from a natural pink color of the ovaries which may be present in control animals or from a generalized redness of the ovaries and surrounding areas, the color of the ovaries should be compared with that of the adjacent coils of the Fallopian tube. If generalized hyperemia exists and the test is *negative*, both ovary and tube will

appear reddened. If the test is *positive*, the ovary will show a greater degree of hyperemia than the tube. This comparison of the color of the ovary with that of the tube has proved the most useful single aid in evaluating the hyperemic response.

Should a questionable hyperemia be observed, it will be found helpful to wait a few minutes and then re-examine the ovaries. The hyperemia is usually more conspicuous when a short time is allowed to elapse after exposure of the ovaries.

Undue manipulation of the ovaries should be avoided since this may result in deceptive coloring or blanching. The ovaries are readily exposed by lifting the attached fatty tissue with forceps.

RESULTS

AGREEMENT BETWEEN THE RAPID RAT TEST AND THE A.Z. TEST

During a twelve-month period, a total of 306 rapid rat tests were performed. The results of these tests were compared with routine Aschheim-Zondek tests performed with the same urines. The findings are summarized in Table 1. Out of a total of 286 A.Z. tests which gave clear-cut positive or negative results, the rapid rat test was in agreement in 278 instances, a percentage agreement of 97.2. Twenty urines produced reactions in the A.Z. tests which were classified as incompletely positive. These responses were characterized by conspicuous distention of the uteri of test animals and patency of the vaginae, but lacked the essential criteria for a positive reaction, i.e., corpora lutea and/or hemorrhagic follicles. It has been our experience that this intermediate-type reaction is obtained when the chorionic gonadotropin titre of the urine is low. These urines gave 12 positive and 8 negative hyperemic responses.

TABLE 1. AGREEMENT BETWEEN THE RESULTS OF THE RAPID RAT TEST AND THE ASCHHEIM-ZONDEK TEST

	Number of Tests	No. of Rat Tests Agreeing with A.Z. Results	Per cent Agreement
Positive A.Z. tests	182	177	97.3
Negative A.Z. tests	104	101	97.1
Total	286	278	97.2

ACCURACY OF THE RAPID RAT TEST AS COMPARED WITH THE A.Z. TEST

In 205 cases it was possible to compare the results of the pregnancy tests with the clinical diagnosis. The accuracy in 194 tests, where either a positive or negative A.Z. test was obtained, is indicated in Table 2. In 11 cases an intermediate-type response was obtained in the A.Z. test and these results are compared with the results of the rapid rat test in Table 3.

TABLE 2. ACCURACY OF THE RAPID RAT TEST AND THE A.Z. TEST IN 194 CASES

Test	Clinical Diagnosis Positive			Clinical Diagnosis Negative			Accuracy for All Tests
	No. of Patients	Correct Diagnosis	Per cent Accuracy	No. of Patients	Correct Diagnosis	Per cent Accuracy	
Rapid Rat	128	126	98.4	66	64	97.0	97.9
Aschheim-Zondek	128	126	98.4	66	66	100.0	99.0

In the group of 128 patients with a clinical diagnosis of pregnancy, an incorrect diagnosis was obtained with both tests in two instances. In no case was an incorrect result given by both tests on the same urine and the results of repeat tests agreed with the clinical diagnosis in every instance.

While no false positive tests were obtained with the A.Z. test, 2 incorrect diagnoses were made in the group of 66 urines from nonpregnant women with the rapid test. The accuracy for the rapid rat test was 97.9 per cent as compared with 99.0 per cent for the A.Z. test.

It has been the authors' experience that a statement of accuracy for any

TABLE 3. RESULTS OF RAPID RAT TEST WITH URINES GIVING INTERMEDIATE-TYPE RESPONSES IN A.Z. TESTS

Clinical Diagnosis	No. of A.Z. Tests with Intermediate Responses	Results of Rat Tests	
		No. Positive	No. Negative
Probable spontaneous abortion	4	0	4
Early pregnancy	1	1	0
Threatened abortion	2	2	0
Incomplete abortion	1	1	0
Early postpartum	1	1	0
Uterine fibromyoma	1	0	1
Bilateral hydrosalpinx and amenorrhea	1	1	0

FIG. 1. Ovaries of animal on right illustrate a positive hyperemic response. The marked reddening of these ovaries is in striking contrast to the pale ovaries of the control animal on the left.

biological pregnancy test requires some qualification. In order to determine the above stated accuracies it was necessary to eliminate those tests in which intermediate-type reactions were obtained with the A.Z. tests. These weak reactions are not uncommon and though the clinical condition of the patient may provide a clue as to the cause for a low chorionic gonadotropin titre, the test result is not a conclusive one. Several conditions which were associated with intermediate-type reactions are listed in Table 3. Similar reactions have occasionally been obtained with urines from postmenopausal and amenorrheic women. When urines which give an intermediate-type reaction in the A.Z. test are used in the rapid test rat the results are variable, in some cases being positive and in others negative. The type of reaction obtained undoubtedly depends in large part upon whether or not there is an adequate amount of the essential gonadotropin in the urine to produce a recognizable hyperemic response.

RELATIONSHIP OF TIME TO THE HYPEREMIC RESPONSE

Zondek and Sulman (6) have emphasized the importance of the time factor in the production of ovarian hyperemia following subcutaneous injections of chorionic gonadotropin. We have attempted to evaluate this factor for tests in which pregnancy urine or chorionic gonadotropin was injected intraperitoneally.

Two dosages (1 I.U. and 20 I.U.) of chorionic gonadotropin were used and tests were allowed to run for intervals of two, four, six and sixteen hours. The response and degree of hyperemia noted in each test animal is tabulated in Table 4. It will be observed that negative responses were obtained in only 3 of the 20 animals used. Two negative responses were obtained in the two-hour group and one in the sixteen-hour group. While all test animals gave a positive response at the end of four hours, the hyperemic response was uniformly more intense in the six-hour group.

In an additional series (Table 5), 2 cc. of pregnancy urine was injected intraperitoneally and the animals were sacrificed at intervals of two, six and sixteen hours. The same dose was administered subcutaneously to a fourth group and the animals were sacrificed at the end of sixteen hours. In the total of 20 animals used, no negative responses were obtained. The least intense hyperemia was observed in the two-hour group, though the heaviest test animal (70 Gm.) of the group showed marked ovarian hyperemia. Approximately the same degree of hyperemia was found in the intraperitoneally and subcutaneously injected animals after sixteen hours.

Following the administration of both chorionic gonadotropin and pregnancy urine, the strongest hyperemic response was obtained most uniformly in the heaviest test-animals (55 to 70 Gm.).

SPECIFICITY OF THE HYPEREMIC RESPONSE

Several types of hormonal preparations were used to determine the specificity of the ovarian hyperemic response. The results, summarized in

TABLE 4. HYPEREMIC RESPONSE TO CHORIONIC GONADOTROPIN AT VARYING INTERVALS OF TIME

Duration of Test	Animal Number	Body Wt. Gm.	Dosage of Chorionic Gonadotropin* I.U.	Response	Intensity of Hyperemia**
	11	36	1	—	0
	12	45	1	+	I
Two hours	13	60	20	+	II
	14	42	20	—	0
	15	43	20	+	I
	6	42	1	+	I
	7	44	1	+	II
Four hours	8	55	20	+	III
	9	44	20	+	II
	10	42	20	+	III
	1	42	1	+	III
	2	43	1	+	III
Six hours	3	60	20	+	III
	4	42	20	+	III
	5	44	20	+	II
	16	46	1	+	II
	17	36	1	+	I
Sixteen hours	18	60	20	+	III
	19	48	20	—	0
	20	42	20	+	I

* Chorionic gonadotropin obtained through the courtesy of The Upjohn Company, Kalamazoo, Michigan.

** The intensity of the hyperemic response was arbitrarily graded from I to III depending upon the redness of the ovaries.

Table 6, indicate the high degree of responsiveness to both chorionic gonadotropin and the pituitary luteinizing hormone. The positive hyperemic response obtained with the higher dosage of FSH was probably due to the presence of a trace of luteinizing hormone.

DISCUSSION

Our primary interest in the hyperemia response of the rat ovary is whether or not it is sufficiently dependable to be the basis for a rapid, routine test for pregnancy. Although Kupperman and Greenblatt (3) have

TABLE 5. HYPEREMIC RESPONSE TO 2 CC. OF PREGNANCY URINE* AT VARYING INTERVALS OF TIME

Duration of Test	Animal Number	Body Wt. Gm.	Response	Intensity of Hyperemia**
Two hours	21	70	+	III
	22	43	+	II
	23	45	+	I
	24	44	+	I
	25	42	+	I
Six hours	31	46	+	III
	32	46	+	III
	33	44	+	II
	34	40	+	II
	35	42	+	II
Sixteen hours (Intraperitoneal injection)	36	42	+	III
	37	46	+	III
	38	64	+	III
	39	42	+	II
	40	44	+	II
Sixteen hours (Subcutaneous injection)	41	44	+	II
	42	46	+	III
	43	45	+	II
	44	42	+	III
	45	65	+	III

* In tests on the immature mouse, the potency of this urine was found to be 1000 M.U. per 1 cc. This concentration is comparable to that found in urines obtained at the peak of chorionic gonadotropin excretion during the first trimester of pregnancy.

** See explanation, Table 4.

reported a high degree of accuracy with a two-hour test, others have obtained less gratifying results. Both Bunde (4) and Zondek and his associates (5) have reported relatively low per cent accuracies with both the two and six-hour tests. The latter group of workers, with subcutaneous injections, have found that only the 24-hour hyperemia test has all the accuracy of the older A.Z. test.

We have altered the technique of Kupperman and Greenblatt to the extent that the test is allowed to run usually between four and six hours rather than confining it to a two-hour interval. The longer intervals provide additional time for the development of hyperemia and at the same time permit the results to be reported on the same day the specimen is received. Following this procedure, an accuracy (97.9 per cent) was obtained which compared favorably with that of the Aschheim-Zondek test.

It appears highly probable that different strains of rats may respond

TABLE 6. HYPEREMIC RESPONSE OF THE OVARY OF THE IMMATURE RAT TO HORMONE EXTRACTS AND NONPREGNANCY URINE

Type of Extract or Urine	Dosage	Hyperemic Response
Follicle stimulating hormone (FSH)*	40 R.U.	Negative
	200 R.U.	Positive
Luteinizing hormone (LH)*	1 R.U.	Negative
	5 R.U.	Positive
Chorionic gonadotropin	1 R.U.	Positive
Nonpregnancy urine: Amenorrhea	25 cc.	Negative
14th day of cycle	2 cc.	Positive
16th day of cycle	2 cc.	Negative

* Fractionated pituitary extracts obtained through the courtesy of Difco Laboratories, Detroit, Michigan. In tests on the immature mouse, the FSH preparation was found to contain traces of LH.

differently to chorionic gonadotropin and that this may account in part for the diverse results reported by different authors. The strain of rats used by Bunde (4) showed a great deal more variability in response and were apparently less responsive to chorionic gonadotropin than that used in the present investigation. This is well illustrated by the fact that no animal in Bunde's series responded to 9.9 I.U. of chorionic gonadotropin and only 2 of 5 animals responded to the injection of 50 I.U. In the present study, only 1 rat in 8 failed to respond to 1.0 I.U. of chorionic gonadotropin. It would be of considerable importance to those contemplating the use of the hyperemia test to determine the responsiveness of their test animals. In the event that marked variability or refractoriness is found, a different source of animals should be sought.

As Zondek and Sulman (6) have recently emphasized, the intensity of the hyperemia response is related to the duration of the test. This relation-

ship was more apparent in the series (Table 4) where relatively small amounts (1 or 20 I.U.) of chorionic gonadotropin were administered. Poorer responses were obtained at two hours than at four or six hours. The response at sixteen hours was little better than at two hours. In view of the likelihood that some urines with a low concentration of gonadotropin will be encountered, it would appear to be desirable to allow at least four hours for routine tests.

It is undoubtedly true, as Zondek and associates (5) suggest, that the hyperemia test is of less value than the A.Z. test in cases of disturbed pregnancy, i.e., ruptured ectopic pregnancy and threatened, incomplete or missed abortion. A low concentration of chorionic gonadotropin, which is not infrequently associated with these conditions, can be detected in the routine A.Z. test and is of diagnostic significance. On the other hand, the hyperemia test may be either positive or negative. In either event, the result is noncontributory or misleading from the clinicians' standpoint. In other instances a doubtful hyperemic response may be obtained, necessitating an inconclusive report.

Another disadvantage of the hyperemia test should be emphasized. Although Kupperman and Greenblatt (3) tested urines from nonpregnant women at different times of the cycle and obtained only negative results, Farris (7) has reported obtaining positive hyperemic responses at about mid-cycle. As indicated in Table 6, the pituitary luteinizing hormone induces ovarian hyperemia at relatively low concentrations. At that time in the cycle when the gonadotropin concentration is high, one might expect to obtain a positive hyperemic response. This might lead to a false diagnosis of pregnancy in some instances where the cycles are markedly irregular.

SUMMARY

The application of the hyperemia response of the rat ovary to chorionic gonadotropin as a routine test for pregnancy is discussed. In 205 tests, an accuracy of 97.9 per cent was obtained as compared with an accuracy of 99.0 per cent for the Aschheim-Zondek test. The tests upon which this accuracy was determined were exclusive of 11 tests which gave incompletely positive A.Z. reactions. Such incomplete reactions are not uncommonly obtained in cases of disturbed pregnancy, such as threatened or incomplete abortion. In these cases the hyperemia test may be positive, negative or inconclusive, depending upon the concentration of chorionic gonadotropin in the urine. The hyperemia test is therefore of little diagnostic value in these conditions.

In experiments with chorionic gonadotropin and pregnancy urine, it was found that the hyperemia was uniformly more intense after an interval of four or six hours than after two hours. There appeared to be no advantage

to an interval of sixteen hours, regardless of whether the urine was injected subcutaneously or intraperitoneally.

Tests with several hormonal preparations indicated a high degree of effectiveness of both chorionic gonadotropin and pituitary luteinizing hormone in producing ovarian hyperemia.

The test animals used in this study were highly responsive to chorionic gonadotropin and showed little variability in response. It is suggested that the lower accuracy experienced by other workers may be due to a relative refractoriness of some strains of rats.

The rapidity and simplicity of the hyperemia test, together with its high degree of accuracy, make it a valuable adjunct to other biological diagnostic procedures.

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ADRENAL CORTICAL HORMONE EXCRETION IN ENDOCRINE AND NONENDOCRINE DISEASE AS MEASURED BY CHEMICAL ASSAY

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IN A previous paper (1) a method was described for the estimation of adrenal cortical steroids in human urine by the quantitative measurement of the formaldehyde liberated by oxidation with periodic acid from urinary extracts. This method has been used in the study of normal subjects and in patients with endocrine and nonendocrine diseases; the results are presented in this paper.

The technique which we have employed measures a chemical property of the urinary steroids possessing a C-17 side chain. Presumably, not only active compounds but also inactive substances are measured. This method, therefore, does not possess the same specificity possessed by bioassay (2, 3) and its validity must rest on how well the results conform to the known pathologic physiology of the adrenal and the results obtained using bioassays. The possibility exists that, because chemical methods measure both active and inactive substances, information may be obtained which is of different significance and would supplement information obtained by bioassay.

The assays to be reported have all been performed with a standardized technique and it is believed that the differences to be reported are significant. The quantitative aspects of the method are under further investigation and relative, rather than absolute values, should be considered.

METHODS

Urine samples were collected for 24 hours using 10 cc. of chloroform to inhibit bacterial growth. The collections were carefully supervised and every effort was made to insure their accuracy. The urine was stored for periods usually not longer than 48 hours at 5° Centigrade until extraction. Extraction and assay were carried out according to the method previously described (1). Results have been expressed in milligrams of formaldehydo-

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genic steroids[1] for 24 hours, calculated on a weight basis equal to 13.1 times that of formaldehyde found (1).

RESULTS

The formaldehydogenic steroid content of urine from normal healthy adults, patients with obesity and patients with essential hypertension. The subjects in these three groups did not present signs or symptoms suggesting adrenal dysfunction and will serve for comparison with the groups to be described later. According to the data presented in Table 1 and in

TABLE 1. EXCRETION OF FORMALDEHYDOGENIC STEROIDS IN SUBJECTS WITHOUT KNOWN ENDOCRINE DISEASE

Subject	Sex	mg./day	Subject	Sex	mg./day	Subject	Sex	mg./day
A. Normal Healthy Adults			B. Obese Subjects			C. Hypertensive Subjects		
W.D.	M	1.5	G.B.	M	1.5	N.P.	F	1.3
W.D.	M	1.2	E.J.	M	1.2	S.	M	1.0
H.J.	M	1.5	P.P.	M	1.4	M.	M	1.1
A.W.	M	1.6	C.D.	M	1.6			
R.W.	M	1.3	M.B.*	F	1.0			
B.S.	M	1.0	S.B.	F	1.1			
B.T.	M	2.1	A.M.*	F	1.1			
B.T.	M	1.9	A.S.	F	1.4			
B.T.	M	1.1	A.S.	F	(5.7)**			
M.H.	F	1.2	A.S.	F	1.0			
M.H.	F	1.0	K.M.	F	1.2			
G.P.	F	1.0	K.M.	F	1.8			
H.D.	F	1.0	A.G.*	F	1.3			
			E.Z.	F	(3.3)**			
			E.Z.	F	1.6			

* These patients had hypertension in addition to obesity.

** These values were obtained during our initial experiences with the test. Since they are not in line with other values obtained in the same patient, we question their accuracy.

Figure 1, the amount of formaldehydogenic steroids excreted by these individuals, with few exceptions, was within the range of 1.0 to 1.6 mg. per day. The normal subject, B. T., excreted on two occasions significantly greater amounts of formaldehydogenic steroids than the other normal

[1] We are not entirely satisfied with this terminology, but we find less objection to it than to others that we have considered: corticosteroids, corticoids, cortin, 20-ketosteroids, or 11-oxysteroids.

subjects. No apparent explanation could be offered for this finding. He had previously had assays performed in the laboratory of Dr. Fuller Albright (4) and excessive quantities of cortin were found by bioassay on one occasion. Two subjects in the obese group, A. S. and K. M., excreted large amounts of formaldehyde-liberating compounds, but this could not be confirmed on repeated assays. We have concluded that these isolated findings could represent technical errors early in our experience.

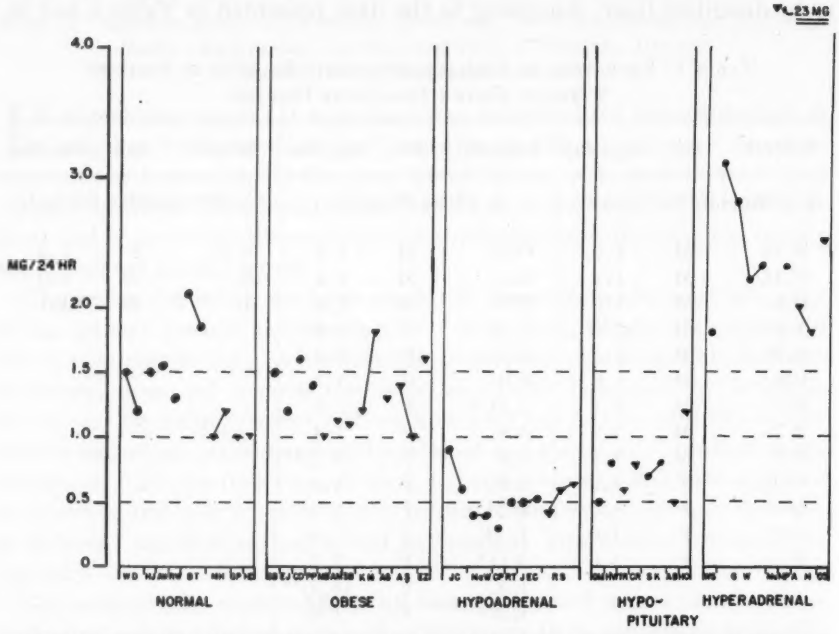

FIG. 1. Excretion of formaldehydogenic steroids in normal and in obese subjects, and in patients with adrenal and pituitary disease. Assays on male subjects are represented by dots and on female subjects by triangles. Multiple assays on the same patient are connected by a solid line. (The broken lines merely help to compare the values in the various columns.)

Hypertension in itself was not observed to be associated with excessive excretion of formaldehyde-liberating material, according to our limited number of observations. The possibility remains that other steroids of adrenal origin, not measured by this technique, are implicated in this disorder.

Excretion of formaldehydogenic steroids in adrenal and pituitary disease. Twelve assays have been performed on the urine from six patients with Addison's disease and the results are presented in Table 2. Except for

the first assay on J. C., all values were between 0.3 and 0.65 mg. per day. These patients all presented the pathognomonic features of the disease. At the time of assay they were all in relatively good nutritional and metabolic state.

The patients with Cushing's syndrome require more explanation. E. M. was a young woman who displayed a full-blown picture of this disease, with

TABLE 2. EXCRETION OF FORMALDEHYDOGENIC STEROIDS IN ADRENAL AND PITUITARY DISEASE

Subject	Sex	mg./day
A. Addison's Disease		
J.C.	M	0.9
J.C.	M	0.6
McW.	M	0.4
McW.	M	0.4
C.P.	M	0.3
R.T.	M	0.5
J.Co.	M	0.5
J.Co.	M	0.5
J.Co.	M	0.4
R.S.	F	0.4
R.S.	F	0.6
R.S.	F	0.65
B. Cushing's Syndrome		
E.M.	F	23.0
G.W.	M	3.1–2.8
G.W.	M	2.2
G.W.	M	2.3
W.S.	M	1.8
C.McL.	F	1.2
		(in remission)
C. Suspected Hyperadrenocorticism		
M.P.	M	2.3
A.M.	F	2.0
A.M.	F	1.8
C.M.S.	F	2.5
D. Adrenogenital Syndrome		
G. (postoperative)	F	1.5
E. Panhypopituitarism		
C.R.	F	0.8
T.R.	F	0.6
G.M.	M	0.5
H.W.	M	0.8
S.K.	M	0.7
S.K.	M	0.8
A.B.	F	0.5
H.O.	F	1.2
F. Acromegaly		
H.	F	1.3

amenorrhea, hirsutism, obesity, cutaneous striae, disturbed carbohydrate metabolism and hypertension. The urine for only one day was available for assay; it contained 23 mg. of formaldehydogenic steroids. The patients G. W. and W. S. present an interesting variant of the syndrome. Each showed the facies of Cushing's disease with a striking distribution of fat about the face and neck. A moderate amount of hypertension was present in each, but carbohydrate metabolism, as measured by glucose tolerance curves and glucose-insulin tolerance curves, was entirely normal. Cutaneous striae were present in G. W., but not in W. S. Osteoporosis was not

severe in either individual. It seems possible that in these two patients the steroids which are active in maintaining blood pressure and electrolyte metabolism were formed to a greater extent than were those that are active in carbohydrate metabolism. This thesis is consistent with the results obtained for excretion of formaldehydogenic steroids, which in each patient was only moderately elevated.

M. P. was suspected of having hyperactivity of the adrenals because of

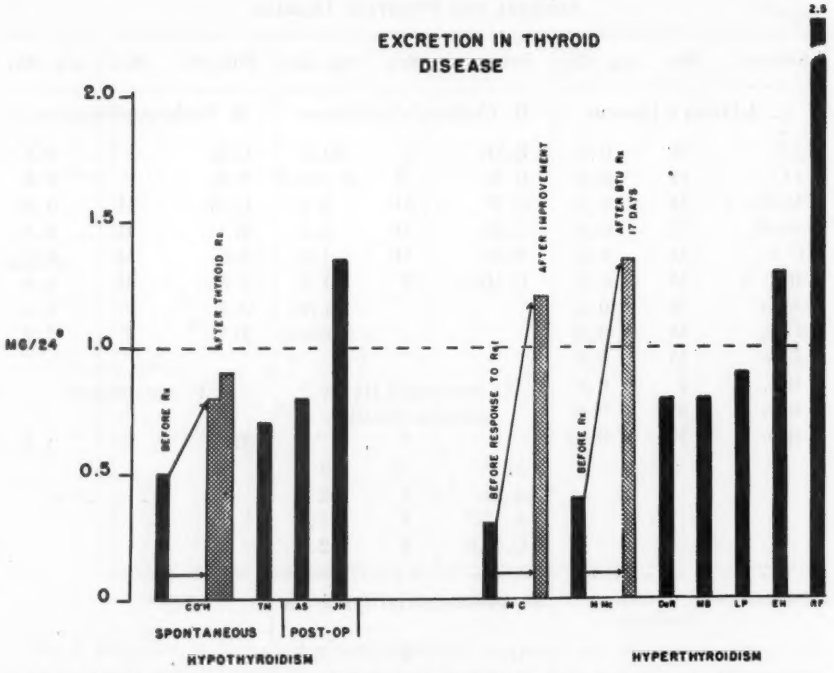

FIG. 2. Note that in both hypothyroidism and hyperthyroidism the excretion of formaldehydogenic steroids tended to be less than 1.0 mg. per day. M. C. was treated with radioactive iodine (I 131). Repeated studies on this patient are shown in Fig. 3. Patient M. Mc. was treated with 6-n-butylthiouracil (BTU).

cutaneous striae, obesity and slight impairment of carbohydrate tolerance. Formaldehydogenic steroid excretion on one occasion was found to be 2.5 mg. per day. A. M. and C. M. S. were also suspected of adrenal hyperactivity because of amenorrhea, changes in fat distribution and slight hirsutism. These patients showed impaired glucose tolerance of the insulin-resistant type. Hypertension was also present. Excretion of formaldehydogenic steroids seemed to be elevated in these two patients.

Formaldehydogenic steroids were excreted in the urine of one patient in normal amounts; C. McL. had had Cushing's syndrome at puberty, but she had undergone a spontaneous and persistent remission. A normal value was also obtained in M.G., a patient with the adrenogenital syndrome, who one year previously had had a masculinizing tumor of the right adrenal removed.

Studies of the excretion of formaldehydogenic steroids have been made

TABLE 3. EXCRETION OF FORMALDEHYDOGENIC STEROIDS IN THYROID DISEASE

Subject	Sex	mg./day
A. Spontaneous Myxedema		
T.M.	F	0.7
C.O'H.	F	0.5
B. Postoperative Hypothyroidism		
A.S.	F	0.8
J.H.	F	1.4
C. Hyperthyroidism		
M.C.*	F	0.3
M.McL.	F	0.4
DeR	M	0.8
DeR.	M	0.8
M.B.	F	0.8
L.P.	F	0.9
E.H.	F	1.3
E.S.	F	0.8
R.F.	M	2.5

* The value recorded occurred during the most severe stage of her disease. See Fig. 3 for additional assays on this patient.

on seven cases of panhypopituitarism. In two patients (C. R. and T. R.) the cause of the disease was postpartum necrosis of the pituitary. Two individuals suffered from chromophobe adenoma (G. M. and H. W.) and the etiology was obscure in three patients (S. K., A. B. and H. O.). The values which were obtained were intermediate between the group with Addison's disease and the normal subjects. In only one individual, H. O., was the excretion within the normal range. No abnormality of excretion of formaldehydogenic steroids was found in the single case of acromegaly.

Excretion of formaldehydogenic steroids in thyroid disease. The results of formaldehydogenic steroid assays on patients with thyroid disease are presented in Table 3 and in Figure 2. Both hypothyroidism and hyperthy-

roidism tended to be associated with decreased amounts of urinary formaldehydogenic steroids. The excretion was more depressed in two cases of spontaneous myxedema than in the two cases of postoperative hypothyroidism. In one patient with spontaneous myxedema excretion of formaldehydogenic steroids returned almost to normal following treatment with desiccated thyroid.

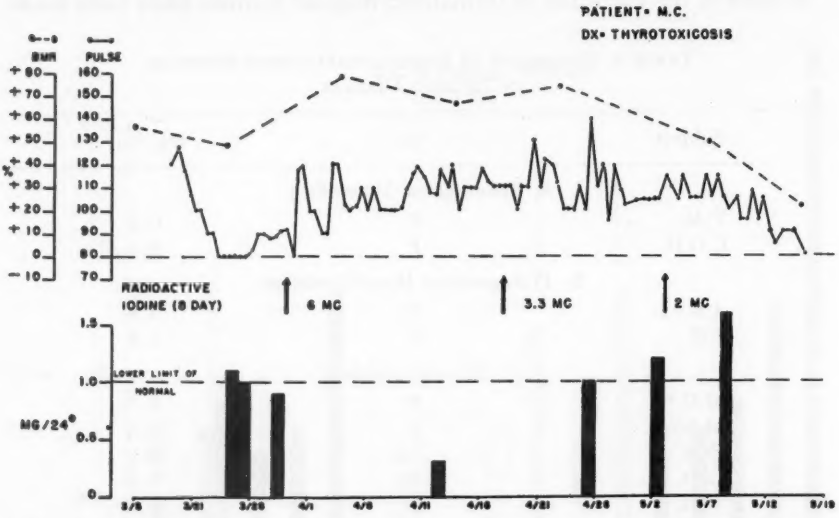

FIG. 3. Excretion of formaldehydogenic steroids in hyperthyroidism treated with radioactive iodine. Note the exacerbation in the disease as evidenced by tachycardia (as well as many other manifestations) produced by the first dose of radioactive iodine.

In two patients with hyperthyroidism the excretion of formaldehydogenic steroids was decreased to levels comparable to that of Addison's disease. In four other individuals the excretion was lower than normal, while in two subjects, normal or excessive excretion of formaldehydogenic steroids was found. In the two patients with the lowest excretion of formaldehydogenic steroids, response to specific treatment (radioactive iodine in M. C. and 6-n-butylthiouracil in M. McC.) was followed by the excretion of normal quantities of formaldehydogenic steroids in the urine.

One of these individuals with hyperthyroidism, M. C., was studied in greater detail and the results are presented in Figure 3. She was a Chinese girl, aged 23 years, who entered the Boston City Hospital with a large thyroid gland and signs of moderately severe hypermetabolism. Improvement followed sedation, bed rest and a good diet with vitamin supplements. Excretion of formaldehydogenic steroids at this time was 1.2, 1.0

and 0.9 mg. per day on successive assays. A severe exacerbation of her disease followed the administration of 6 millicuries of radioactive iodine (I 131). She developed marked tachycardia, palpitation, dyspnea, restlessness, edema of the ankles, weakness and tightness in the neck. There was also an increase in the basal metabolic rate. During this period of mild thyroid storm the excretion of formaldehydogenic steroids decreased to

TABLE 4. EXCRETION OF FORMALDEHYDOGENIC STEROIDS IN SCURVY

Date	mg./day	Remarks
		Patient 1, W.C., Male aged 64 years.
4 Feb. 1947	0.85	Prior to treatment Vitamin C, 1 Gm. per day by mouth begun on Feb. 6, 1947.
16 Feb. 1947	0.5	
23 Feb. 1947	0.8	
3 Mar. 1947	1.8	
11 Mar. 1947	5.0	
29 Mar. 1947	2.9	
30 Mar. 1947	2.8	
		Patient 2, M.McC., Female aged 64 years.
6 Mar. 1947	1.0	Prior to treatment Vitamin C, 1 Gm. per day by mouth begun on Mar. 7, 1947
12 Mar. 1947	0.4	
11 Apr. 1947	1.0	
14 Apr. 1947	0.8	
22 Apr. 1947	0.9	
		Patient 3, J. McK., Male aged 30 years.
2 May 1947	0.4	Treatment started before assay.
6 May 1947	0.2	(see Figure 5)
9 May 1947	0.3	
11 May 1947	0.4	
16 May 1947	1.0	

0.3 mg. per day (checked). When the therapeutic effect of radioactive iodine became evident, formaldehydogenic steroids reappeared in normal amounts in the urine.

Excretion of formaldehydogenic steroids in vitamin C deficiency. Because of the high concentration of vitamin C in the adrenal and the recent chemical (5) and physiological studies (6) suggesting an important role in adrenal function, we have studied the excretion of formaldehydogenic steroids in three cases of clinical scurvy. All three patients had a dietary history suggesting grossly inadequate vitamin C intake; W. C. because of

a self-imposed diet for peptic ulcer, M. McC. because of poverty and poor food habits and J. McK. because of severe alcoholism. Deficiencies of other vitamins undoubtedly existed, but their manifestations were not definite. Subcutaneous ecchymoses and perifollicular hemorrhages were present on

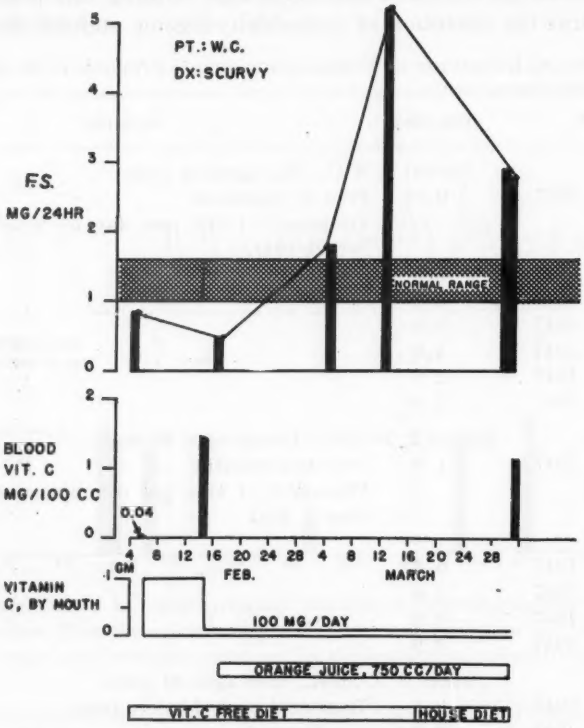

FIG. 4. Excretion in urine of formaldehydogenic steroids (F. S.) in scurvy, patient 1, W. C.

the legs of all three patients when they entered the Boston City Hospital. The tourniquet test was positive in each case. Analyses of the vitamin C content of whole blood in M. McC. and W. C. indicated that less than 0.06 mg. per 100 cc. was present.

Excretion of formaldehydogenic steroids in W. C. before treatment was begun was 0.9 mg. per day. Vitamin C was then administered in a dose of 1 gram a day. Eleven days later the excretion of formaldehydogenic steroids was 0.5 mg. per day and five days thereafter it was 0.8. Later, excessive amounts of formaldehydogenic steroids were found in the urine. The results are presented in Table 4 and Figure 4.

The results of assays on M. McC. are also presented in Table 4. The excretion of formaldehydogenic steroids prior to treatment was 1.0 mg. per day. Following 1 gram of vitamin C daily for 6 days, the excretion of formaldehydogenic steroids had decreased to 0.4 mg. One month later the excretion had risen to more nearly normal levels. No hypersecretory phase was noted, perhaps because of the unequal spacing of the urinary assays.

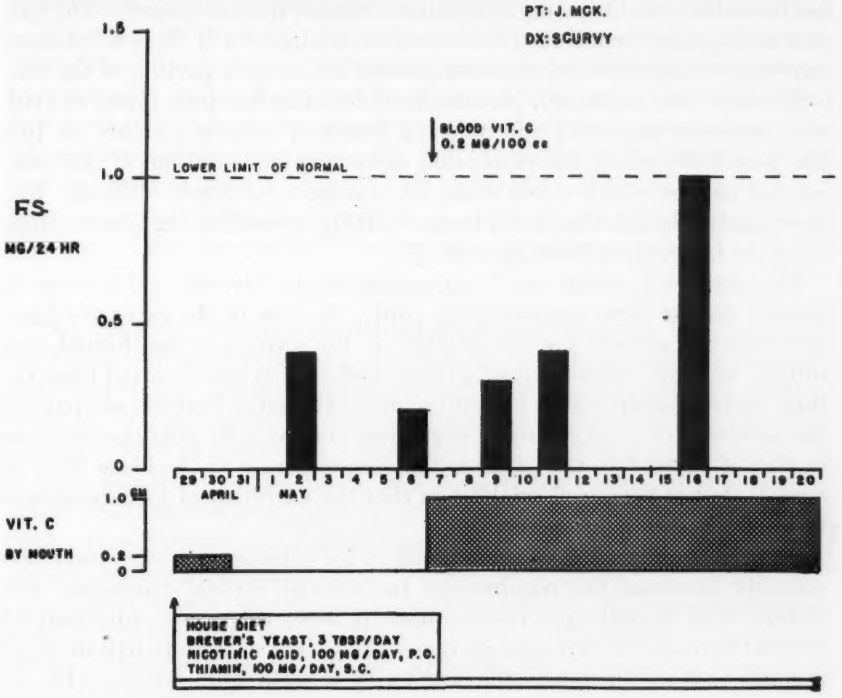

FIG. 5. Excretion in the urine of formaldehydogenic steroids (F. S.) in scurvy, patient 3, J. McK.

The third patient, J. McK., was a man, aged 30, who had been on a long alcoholic debauch and he had eaten very irregularly for several months. Treatment with vitamin C had been started before studies of formaldehydogenic steroids could be started. Subsequent assays of urinary formaldehydogenic steroids are presented in Figure 5 and Table 4. On four separate occasions excretion of formaldehydogenic steroids was below 0.4 mg. per day. It was not possible to make extended observations on this patient. Despite the low excretion of formaldehydogenic steroids in the urine, there were no symptoms or laboratory findings suggesting adrenal insufficiency; in fact the patient showed remarkable improvement.

DISCUSSION

In patients with adrenal disease the quantity of formaldehydogenic steroids excreted in the urine is consistent with the known pathologic physiology of these conditions and confirms reports using bioassay (7) and chemical assay measuring the reducing power of urinary steroids (8, 9, 10). It was found that patients with Addison's disease excreted significantly less formaldehyde-liberating compounds than did normal subjects. The fact that such patients continued to excrete small amounts of these substances warrants consideration of an extra adrenal source for a portion of the material which was measured. An analogous situation has been found to exist with methods measuring the reducing power of urinary steroids (8, 10). The possibility exists, therefore, that there may be variations of this non-adrenal portion which would make interpretation of results difficult. Unfortunately, facilities have not been available to confirm our observations using the bioassay of Venning et al. (3).

The changes in excretion of formaldehydogenic steroids which occur in thyroid disease have received little study. In view of the extensive literature relating adrenal size and function to the activity of the thyroid, this subject warrants careful investigation. Talbot (10) has reported observations on two patients with hypothyroidism. He found that in each patient the excretion of 11-oxysteroids was below normal; following the administration of thyroid to one of them the excretion rose to the lower limit of normal. It has been well established that the excretion of 17-ketosteroids may be very low in myxedema (11).

Hyperthyroidism places the patient under considerable stress and presumably increases the requirement for adrenal cortical hormones. The results from animal experiments make it seem clear that administered thyroid hormone or thyroxine increases the weight of the adrenals in intact animals (12) and increases the sensitivity to potassium toxicity (13). In dogs, at least, adrenal cortical hormone appears to counteract the deleterious effect of thyroxine on nitrogen metabolism (14). Because of the possibility of adrenal exhaustion in "thyroid storm," adrenal extracts have been advocated and used with results which are difficult to interpret (15). The excretion of 17-ketosteroids has been found to be decreased in thyrotoxicosis (11), but little direct evidence has been obtained concerning cortin excretion in this disease. Venning and Browne (7) have reported an isolated observation on a young man with moderately severe hyperthyroidism. Normal excretion of corticoids was found. Our studies of this condition show that there is commonly a slight decrease of excretion of formaldehydogenic steroids, despite the stress imposed by the disease. In some patients with severe hyperthyroidism the cortin excretion was quite low. We have had an opportunity to make assays on one patient, M. C., before,

during and following a mild thyroid storm. A decreased excretion coincided with the most severe phase of the disease.

The reason for the low urinary excretion of formaldehydogenic steroids despite the biological evidence of adrenal hyperactivity remains obscure. It seems possible that the destruction of hormone should be increased by the greatly augmented rate of biological oxidation. In this instance urinary excretion would not represent hormone production.

The observations which we have made on scurvy are also difficult to interpret. Contrary to our expectation, decreased urinary excretion seemed to occur *after*, rather than prior to, vitamin C treatment. McKee, Cobbey and Geiman (16) have recently demonstrated a remarkable impotence of adrenal cortical steroids to raise the level of glycogen in the livers of fasting scorbutic guinea pigs. Whether the administration of vitamin C to patients with scurvy may aid in the formation of a steroid-ascorbic acid complex (5) and in some way prevent excretion remains to be determined.

SUMMARY

The urinary excretion of formaldehydogenic steroids has been estimated by a chemical method based on the liberation of formaldehyde from urinary extracts by treatment with periodic acid. Presumably, this method measures nonglycogenic as well as glycogenic steroids and is, therefore, not considered to be directly comparable with tests of gluconeogenesis. It is very desirable to compare the two types of tests in order to determine which one indicates best the activity of the adrenal glands. It is probable that neither method yields more than a rough indication of the quantity of adrenal hormones manufactured.

In a small number of normal persons the excretion of formaldehydogenic steroids was found to be from 1.0 to 1.6 mg. per 24 hours. In all but one of eleven determinations on 6 patients with Addison's disease the values were less than 0.65 mg. per day.

In 7 patients with panhypopituitarism the excretion was below normal in all but one patient who excreted 1.2 mg. per day. Increased excretion occurred in patients with Cushing's syndrome.

Hypothryoidism and hyperthyroidism were associated with decreased excretion of formaldehydogenic steroids.

The administration of vitamin C to three adult patients with scurvy was associated with a decrease in the excretion of formaldehydrogenic steroids, followed by an increase, which was above normal in one instance.

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RELATION OF OBESITY TO THE FUNCTION OF THE THYROID GLAND, ESPECIALLY AS INDICATED BY THE PROTEIN-BOUND IODINE CONCENTRATION IN THE PLASMA

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IN 1916 Means (1) reported that even in patients with marked obesity the basal metabolic rate was usually normal. Since then much evidence has accumulated (2) to indicate that hypothyroidism does not play an important role in the etiology of obesity, but this interrelationship has not been clarified completely. In individuals with myxedema there does not tend to be much adiposity. For example, Boothby and Sandiford (3) found in 81 per cent of 94 obese patients that the basal metabolic rate was within 10 per cent of normal. Strouse, Wang and Dye (4) observed that the basal metabolic rate of normal persons was similar, per square meter of body surface, to those of subjects who were overweight. Grafe (5) found a definite decrease in the basal metabolic rate in only 3 of 180 patients with marked obesity. In most of the obese subjects whom we studied (6) the basal metabolic rate was normal.

MacKay and Sherrill (7) observed that several months after total thyroidectomy of rats 170 days old there was a much less fat content than in the control animals. Plummer (8) found that only a small proportion of 200 patients with myxedema were significantly obese. Moreover, he observed that when the excess fluid was eliminated, many of the subjects were underweight.

Since the concentration of the protein-bound iodine of the plasma is generally a better indicator of the rate of production of thyroxin than is the test of the basal metabolic rate, determinations of the former, as well as the latter, were made in 24 obese individuals.

MATERIAL AND METHOD

Only markedly obese subjects and ones who had had no iodide for more than six weeks were investigated. None of the patients had any symptoms or physical signs suggesting hypothyroidism. All of the patients were ambulatory and were in good health, except for one with hypertension and

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one with diabetes mellitus. Eighteen of the individuals were in their fourth or fifth decade; 5 were males and 19 were females.

Plasma samples were collected using the precautions outlined by Salter (9). The amount of protein-bound iodine was determined, in 17 cases, by the method of Riggs and Man (10). The plasma from the other 7 patients was analyzed in Dr. Salter's laboratory, using the method which he developed (11). Estimations of the basal metabolic rate were made on approximately the same date as blood was drawn for the iodine determination.

RESULTS

The protein-bound iodine of the plasma was found to range from 1.3 to 8.0γ per 100 cc. of plasma (Fig. 1). The question now arises as to what

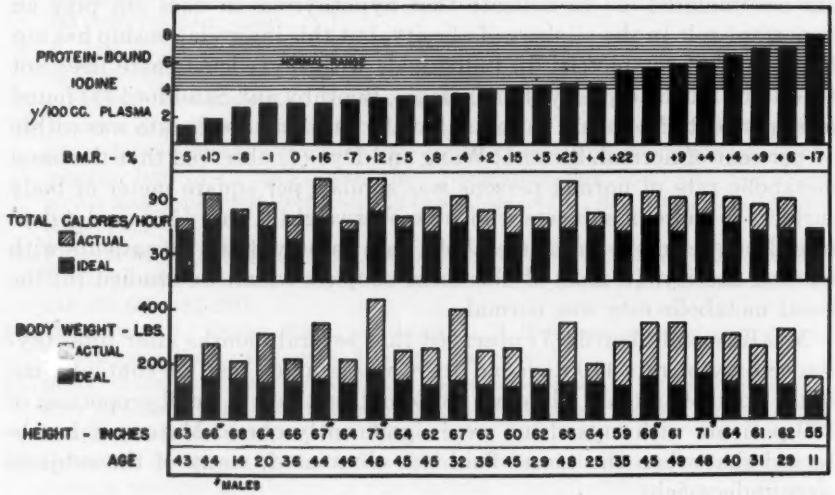

FIG. 1. Note that the concentration of protein-bound iodine was less than 4γ per 100 cc. of plasma in 11 cases. The total oxygen consumption per hour was greater in each instance than that of normal individuals of the same height.

are the limits of the normal range. Relatively few values have been reported on entirely normal individuals, but a moderate number are given for patients with euthyroidism (12). Although exceptions occasionally occur, values from 4 to 8γ per 100 cc. may be considered normal. Winkler (13) has recently presented values for 235 control subjects which ranged from 1 to 15γ per 100 cc. of serum with the majority between 3 and 8γ. Upon comparing the control values obtained by Winkler with the ones which we obtained in the obese subjects, it is observed (Fig. 2) that a greater proportion of the latter group had low concentrations of iodine in

the plasma. In 11 of the 24 patients whom we studied there was less than 4γ; in 5 of the 7 sera analyzed in Dr. Salter's laboratory there was less than 4γ. In only 8 of the 24 patients was the iodine concentration greater than 4.5γ; the highest value was 8.0. There was no significant difference clinically between the patients with hypoiodinemia and the others.

In 18 subjects the basal metabolic rate was in the range between plus 10 and minus 10 per cent. In only one case was it below minus 8, in which instance it was minus 17 per cent. In 5 patients it was between plus 10 and plus 25 per cent, but three of these subjects were dyspneic when they were in the recumbent position.

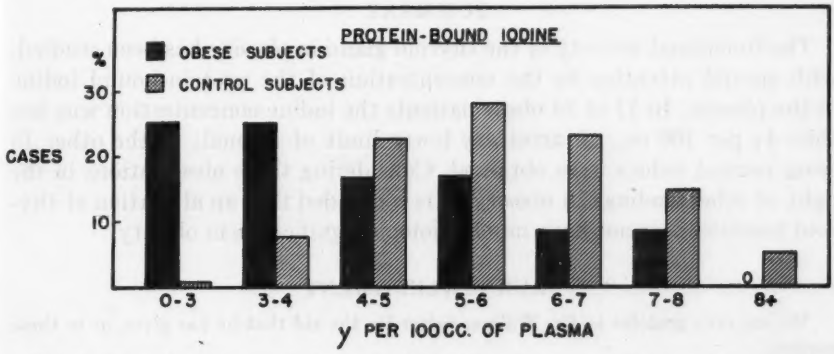

FIG. 2. Note that the protein-bound iodine of the plasma was decreased in a greater proportion of 24 obese subjects than was found by Winkler (13) in 235 control individuals.

As shown in Figure 1, the total number of calories utilized per hour by each patient, in a basal state, was distinctly greater than in individuals of the same height but of ideal weight. There is no correlation of the protein-bound iodine of the plasma with the basal oxygen consumption nor with the amount of obesity or the age of the patient. It can be observed in Figure 1 that there was pronounced obesity in all of the subjects; the average heights were essentially the same as in normal subjects.

DISCUSSION

It is difficult to evaluate the significance of the relatively low concentrations of protein-bound iodine. It is believed that they are technically accurate since (a) most of the determinations were conducted in the same manner as has been found satisfactory in this laboratory for several years, and (b) the results obtained in Dr. Salter's laboratory were comparable to the ones acquired in the Thorndike Laboratory. If hypoiodinemia is of significance in the etiology of obesity, it must play a role in only part of

the patients, although they were similar clinically. Moreover, even though hypoiodinemia had been found in all cases, it might not have been of very much significance etiologically, since patients with myxedema consistently have hypoiodinemia without much obesity. Furthermore, there was not sufficient clinical evidence to diagnose hypothyroidism in any case. Three of the patients were treated for several weeks with 2 grains or more daily of desiccated thyroid without much loss in weight. In 15 obese patients examined postmortem, the thyroid gland was found to be normal macroscopically and microscopically.

Other etiologic factors in obesity are discussed in a separate report (6).

SUMMARY

The functional activity of the thyroid gland in obesity has been studied, with special attention to the concentration of the protein-bound iodine in the plasma. In 11 of 24 obese patients the iodine concentration was less than 4γ per 100 cc., an arbitrary lower limit of normal; in the other 13 cases normal values were obtained. Considering these observations in the light of other findings in obesity, it is concluded that an alteration of thyroid function does not have much etiologic significance in obesity.

ACKNOWLEDGMENT

We are very grateful to Dr. William Salter for the aid that he has given us in these studies.

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STUDIES ON ANTIHORMONE SPECIFICITY WITH PARTICULAR REFERENCE TO GONADOTROPIC THERAPY IN THE FEMALE*

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PROTRACTED administration of extracts containing gonad-stimulating hormones will frequently result in the formation of antihormones (1). With the appearance of these hormone inhibitors two choices present themselves: either to discontinue therapy and await the disappearance of the antihormones, which usually is a matter of 2 to 3 months, or to administer a gonadotropin from another source.

Whether or not a response to another gonadotropic extract in the presence of antihormones can be anticipated has received little clinical consideration. Animal experiments, however, provide leading information and while these studies are too extensive to review here, a good summary in table form is presented by Zondek and Sulman (2). In general, in the rabbit, pregnant mare serum gonadotropin will form antihormones which are specific in their antagonistic action. Sheep pituitary extracts, on the other hand, elicit antigonadotropins which are nonspecific in that they will antagonize the gonadotropic activity of human, ox, horse, pig, rat and rabbit pituitaries, of human chorionic gonadotropin and of pregnant mare serum. Numerous reports are also listed by Zondek and Sulman (2) to show that human chorionic gonadotropin will form antihormones in the rabbit and that these sera are capable of inhibiting human pituitary activity. It is evident from the animal experiments that the source of the gonadotropic extract is a factor in the problem of antihormone specificity. One might, of course, consider the results as being due to extracts which for the most part were not prepared for clinical use. This does not appear to be a determining factor, however, as it has been shown that the clinically used combination of sheep anterior pituitary extract and human chorionic gonadotropin (Synapoidin) will form antigonadotropins in the rabbit and that the serum will inhibit the gonadotropic activity of pregnant mare serum, human chorionic gonadotropin, human pituitary and rat pituitary (3).

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In general, hormones of homologous source do not form hormone antagonists and therefore human chorionic gonadotropin may elicit antihormones in the rabbit but not in man. Furthermore, inhibitory substances against chorionic gonadotropin, human pituitary or sheep pituitary extract do not appear in the serum of postpartum women (4). A recently reported exception must be noted, however, as Segaloff and Parson (5) have presented a case in which inhibitors against chorionic hormone were detected.

Equine gonadotropin (pregnant mare serum) can form antigonadotropins in the human but the inhibitory action appears to be hormone specific in agreement with animal experiments. Rowlands and Spence (6) and later Ostergaard (7) found that anti-PMS serum would not inhibit the gonadotropic activity of human pituitary or human chorionic gonadotropin. Jailer and Leathem (8) could not inhibit the gonadotropic activity of sheep anterior pituitary extract plus human chorionic gonadotropin (Synapoidin) with the serum from a patient showing considerable anti-PMS activity.

Pituitary extracts from animals will form antihormones in man but no information is available to ascertain whether the antagonistic action is specific for the hormone injected or is aspecific to the point of counteracting the secretions from the patient's own pituitary. The current investigation is a beginning study of that problem.

Patients suffering from amenorrhea, sterility or functional menstrual disorders were the cases under study. All were carefully selected and gonadotropins administered because the laboratory findings suggested a deficiency of pituitary gonadotropins. The hormones used were pregnant mare serum (Gonadogen), a combination of sheep anterior pituitary extract and human chorionic gonadotropin (Synapoidin) and human chorionic gonadotropin (Antuitrin-S).[1]

The presence of antigonadotropins in the serum was determined by estimating the degree to which the serum inhibited the ovarian weight increase anticipated with the gonadotropin alone. For these tests, twenty-two day old female rats or mice were used. All animals received the designated dose of gonadotropin and 2 of the 4 littermates received in addition, at another site, a total of 0.9 cc. of the serum to be tested. Injections were made subcutaneously once daily for three days and the animals were killed twenty-four hours after the last injection. The weights of the ovaries and uteri were obtained at autopsy. Pregnant mare serum, human cho-

[1] Grateful acknowledgment is made to Dr. M. H. Kuizenga of the Upjohn Co. for the Gonadogen, to Dr. D. A. McGinty for the Synapoidin and Antuitrin-S, and to Dr. R. Bates of E. R. Squibb & Sons for the Equine Pituitary Gonadotropin.

rionic gonadotropin, human pituitary, equine pituitary and a combination of sheep anterior pituitary extract plus chorionic gonadotropin were all used to determine serum specificity. The water soluble portion of acetone-dried human pituitaries was used.

The specificity of pregnant mare serum antihormones was studied in 2 cases. Each of these patients had been previously treated with another gonadotropin but the resulting antihormones for pregnant mare serum (PMS) were specific. *Case 1* was a sterile patient who had received 15 R.U. of "Synapoidin" three times weekly during two weeks of each month for a four month period. Pregnant mare serum was then administered during a

TABLE 1. INHIBITORY ACTION OF PREGNANT MARE SERUM ANTIHORMONES IN A STERILE WOMAN (Case 2)

Hormone (Total Dose)	Test Animal. Ovarian Weight (mg.)		Test
	Hormone Only	Hormone and Patient's Serum	
Pregnant Mare Serum (10 I.U.)	11.3	3.4	Positive
"Synapoidin" (3 R.U.)	7.4	9.0	Negative
Human Pituitary (2 mg.)	53.0*	54.0	Negative

* Tested in rats, others in mice.
Ovarian weight of control rats, 12.3 mg.; of mice, 3.1 mg.

two week period (total dose, 2400 I.U.) and her serum tested three weeks later. Her serum exhibited a marked anti-PMS effect but did not alter the action of "Synapoidin." *Case 2* was a sterile patient that had received several courses of injections with chorionic gonadotropin followed by two months of twice weekly injections with pregnant mare serum (total dose, 2000 I.U.). Her serum was tested two months after the last injection and a definite inhibitory action against PMS was demonstrated but the same serum did not alter the ovarian stimulating action of "Synapoidin" or human pituitary in the test animal (Table 1).

Case 3. This patient, (G.), suffered from secondary amenorrhea. She had received extended treatment with the combination of sheep AP and chorionic gonadotropin (Synapoidin) prior to the administration of pregnant mare serum for two months. Shortly thereafter her serum was found to be antagonistic to PMS and to human chorionic gonadotropin but was not inhibitory against "Synapoidin" (Table 2). It would seem that PMS antihormones were not specific in this case and indeed the data do not prevent

this conclusion. However, "Synapoidin" is an extract containing chorionic gonadotropin and sheep anterior pituitary and, as will be presented, its antigonadotropins are not specific. Since this patient received "Synapoidin" for an extended period, either the chorionic gonadotropin which it contained stimulated the development of antihormones or nonspecific antagonists to the sheep protein developed, which were more sensitive to chorionic hormone than to the administered hormone combinations.

The nonspecific antihormones developed following the administration of pituitary extracts are well known and during the course of the clinical studies with "Synapoidin" several rabbits were also injected. The rabbit

TABLE 2. ANTIHORMONE TESTS ON PATIENT G. (Case 3) AFTER TREATMENT WITH A COMBINATION OF SHEEP ANTERIOR PITUITARY PLUS HUMAN CHORIONIC GONADOTROPIN (SYNAPOIDIN), AND WITH PREGNANT MARE SERUM (GONADOGEN)

Hormone (Total Dose)	Test Animal. Ovarian Weight (mg.)		Test
	Hormone Only	Hormone and Patient's Serum	
Pregnant Mare Serum (10 I.U.)	57	15	Positive
Chorionic Gonadotropin (20 I.U.)	4.5*	2.5*	Positive
"Synapoidin" (3 R.U.)	36	39	Negative

* Tested on mice.

serum was soon found to be antagonistic to these gonadotropins and the finding that human pituitary extract was also antagonized by this serum prompted a more complete study of our clinical material. Patients were treated with "Synapoidin" from two to three times weekly in 15 R.U. (1 cc.) intramuscular doses during the first two weeks of each month. After from two to five months of therapy the sera failed to contain antihormones for "Synapoidin," but the possibility that substances inhibitory to gonadotropins from other sources might be present, while unlikely, was not eliminated. Therefore, a number of these sera were also tested against human chorionic gonadotropin, pregnant mare serum, equine pituitary and human pituitary and with but one exception were found to be negative (Table 3). The exception was a patient tested after three months of treatment, during which time the "Synapoidin" did not form hormones against itself but did elicit the formation of substances capable of antagonizing the action of chorionic gonadotropin. Although it is not possible to say which of the components of "Synapoidin" was responsible for this antihormone

development it does seem logical to assume that the sheep protein formed nonspecific substances which were more potent against chorionic gonadotropin. A similar situation occurred in the previously discussed *case 3* in which PMS was also administered. It is apparent that PMS is not needed to stimulate the development of antihormones for chorionic gonadotropin when "Synapoidin" is administered. The clinical response was clearly demonstrated in cases of functional menstrual disorders which, for the most part, were restored to normality.

After six months of treatment with "Synapoidin" several patients developed substances in their serum which were capable of reducing the

TABLE 3. ANTIHORMONE TESTS WITH SERA FROM PATIENTS TREATED FOR TWO TO FIVE MONTHS, WITH A COMBINATION OF SHEEP ANTERIOR PITUITARY AND HUMAN CHORIONIC GONADOTROPIN (SYNAPOIDIN)

Hormone	No. of Patients Tested	No. of Positive Tests	Test Animal. Ovarian Weight (mg.)		Test
			Hormone Only	Hormone and Patient's Serum	
"Synapoidin"	12	0	42	47	Negative
Chorionic Gonadotropin	6	1	42*	23*	Positive
Pregnant Mare Serum	3	0	39	47	Negative
Equine Pituitary	4	0	30	30	Negative
Human Pituitary	3	0	45	46	Negative

* Data of the one positive test. Others are averages of all tests.

effectiveness of these gonadotropins. In 4 cases the presence of antihormones was reflected by the failure of the patient to respond to previously successful therapy and by the antihormone tests which were positive for the gonadotropic material administered. Furthermore, the partially nonspecific nature of these antigonadotropins was shown by the inhibitory action of the serum against human chorionic gonadotropin and pregnant mare serum but the lack of such action against human pituitary (Table 4).

These studies recall the antihormonic tendencies of gonadotropins with the general exception of chorionic gonadotropin. When the hormone source is pregnant mare serum the antihormones which develop are specific and consequently a clinical response could be anticipated with a pituitary extract in the presence of anti-PMS serum. The extract would possibly have to be from a source other than horse pituitary. "Synapoidin" while showing no evidence of antihormone formation for the first six months of therapy in the doses used may, on a rare occasion, exhibit antichorionic

hormone action during this time. More extended use of "Synapoidin" can elicit nonspecific antihormone formation, and a response to pregnant mare serum hormone in the presence of "anti-Synapoidin" serum would not be anticipated. Unlike the animal experiments, human "anti-Synapoidin" sera do not inhibit the gonadotropic action of human pituitary. It is necessary to emphasize, however, that continuous administration of this gonadotropin might form substances antagonistic to human pituitary but it is apparent that antihormone formation can be detected before such action is possible and serves as a definite reason to discontinue this

TABLE 4. INHIBITORY ACTION OF HUMAN "ANTI-SYNAPOIDIN" SERUM

Hormone	No. of Patients Tested	No. of Positive Tests	Test Animal. Ovarian Weight (mg.)		Test
			Hormone Only	Hormone and Patient's Serum	
"Synapoidin"*	4	4	57	24	Positive
Chorionic Gonadotropin	2	2	39	18	Positive
Pregnant Mare Serum	4	3	38	16	Positive
Human Pituitary	3	0	36	39	Negative

* Sheep anterior pituitary and human chorionic gonadotropin.

therapy. These data further emphasize the importance of antihormone tests in patients receiving extended gonadotropin therapy.

SUMMARY

Further evidence is presented to support the contention that antihormones which develop following the administration of pregnant mare serum are hormone specific. On the other hand, patients who develop antihormones for a combination of human chorionic gonadotropin and sheep anterior pituitary extract (Synapoidin), have sera of a partially nonspecific nature. These sera will counteract the activity of chorionic gonadotropin and pregnant mare serum but will not affect that of human pituitary. In general, if the sera of patients receiving "Synapoidin" therapy fail to show the development of antihormones for the administered extract, they will also fail to show antagonism to gonadotropins from other sources. It appears that pregnant mare serum therapy would be to no avail in the presence of the formation of "anti-Synapoidin" serum by the patient; but that "Synapoidin" therapy would be effective in the presence of anti-PMS serum.

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ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

The program of the meeting will appear in either the April or May issue of the Journal, and the abstracts of papers presented at the annual meeting will be published in the July issue.

ANNOUNCEMENT OF THE 1948 LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the A.A.A.S. will meet in 1948 at the Forest Hills Hotel, Franconia, New Hampshire, from September 13th to 18th inclusive. The program (which will be published in full at a later date) will consist of four sections:

I. The metabolism of steroid hormones *in vivo* and *in vitro.*
II. Thyroid physiology and function.
III. The role of hormones in tissue and body metabolism.
IV. Hypothalamic neuro-humoral relationships.

Because of limited accommodations, attendance is by invitation, but the Committee on Arrangements will receive applications for membership until June 15, 1948. Applications should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.